RESEARCH PAPER

Formulation and In-Vitro Evaluation of a Nanoemulsion-Based System for Transdermal Drug Delivery

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Abstract

Many therapeutic agents have poor aqueous solubility, fast liver metabolism and low bioavailability, which are long-standing challenges for effective oral drug delivery. Transdermal delivery is a promising alternative due to the advantages of avoiding first pass metabolism and enhancing permeation of the drug across the stratum corneum by nanoemulsion based system. In this investigation, a novel nanoemulsion-based transdermal delivery system to deliver benidipine hydrochloride as a model drug was developed and evaluated. Pseudo-ternary phase diagrams were used to determine the best concentration of each component with isopropyl myristate as oil phase, Tween 80 and 20 as surfactant and propylene glycol and glycerol as cosurfactants. The influence of oil phase content and the composition of cosurfactants on droplet size, polydispersity index, zeta potential and drug loading were thoroughly studied. Physicochemical characterization included dynamic light scattering, thermodynamic stability studies by centrifugation and heating-cooling methods and in-vitro drug diffusion studies by dialysis membrane method. Morphology was assessed by transmission electron microscopy (TEM) and the long-term stability was evaluated after 6 months at ambient temperature. The optimized formulation showed average droplet size in the range of 48.3–82.4 nm and PDI value between 0.26 and 0.29 which indicated the homogeneous size distribution appropriate for transdermal penetration. The content of the drug was well maintained at the level of 99.3 to 99.6% throughout the study period. The nanoemulsion system was found to be able to retain the solubility of the drug in physiological pH and was found to possess sustained in-vitro release characteristics. The results indicate that the nanoemulsion based transdermal systems are promising delivery systems for poorly soluble pharmaceutical compounds.

Keywords: Nanoemulsion; Transdermal Drug Delivery; Benidipine Hydrochloride; Drug Permeation; In-Vitro Evaluation

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1. Introduction

The main drawbacks of conventional oral drug administration are the fundamental limitations affecting therapeutic effectiveness. Some 40% of new pharmaceutical compounds developed have poor water solubility, thereby limiting their bioavailability and usefulness in clinical settings. In addition to solubility problems, drugs with high hepatic first-pass metabolism are not as effective orally and may be more likely to have adverse effects as a result. The gastrointestinal degradation also results in instability of acid-labile or enzyme-sensitive molecules (Zheng et al., 2010). Transdermal drug delivery overcomes these drawbacks and offers direct access to the systemic circulation without the gastrointestinal and hepatic barriers. The outermost layer of the epidermis, called the stratum corneum, is the major barrier to percutaneous drug absorption. This lipid-based barrier allows lipophilic molecules to pass through, but it will not allow in hydrophilic molecules. To overcome this barrier, nanoemulsions, which are colloidal dispersions of droplets ranging from 20 to 200 nanometers, provide advanced solutions. They have small droplet size which has a dramatic effect on the drug solubilization capacity and the permeation kinetic of the drug through the skin (Souto et al., 2022).

Figure 1: Enhancing Drug Delivery with Nanoemulsions

Formulation components have a large impact on nanoemulsion performance. Drug loading and interaction with the stratum corneum is a function of oil phase composition. Isopropyl myristate (IPM) is very effective in solubilizing and has proven acceptable for transdermal use. LCTs help the lymphatics to absorb them without going through the liver and MCTs are more efficient at emulsification. The choice of surfactant is a compromise between emulsifying power and dermal safety: nonionic surfactants are less toxic than ionic types. Cosurfactants enhance penetration while maintaining thermodynamic stability. Ideal droplets are small enough (usually less than 100 nanometers) to penetrate skin spontaneously. Polydispersity index is an indicator of the uniformity of the size distribution; the smaller the value, the more uniform the size distribution of the population and the better the stability will be. The magnitude of Zeta potential predicts the colloidal stability, and a magnitude greater than ±30 millivolts (mV) ensure electrostatic repulsion between the droplets to prevent droplet aggregation (Yadav et al., 2023). Benidipine hydrochloride is an ideal model for such an investigation. Its lipophilicity, low aqueous solubility and high first-pass metabolism, are all the characteristics that face the bioavailability problems nanoemulsion-based transdermal delivery overcomes. The current formulations have different absorption and are not consistently therapeutic. Systematic assessment of formulation factors, stability indicators, and in-vitro performance will provide key scientific data to guide rational formulation and aid in future clinical translation (Callender et al., 2017).

2. Literature Review

Nanoemulsion Technology and Transdermal Drug Delivery

Nanoemulsions are highly advanced colloidal systems with special thermodynamic and kinetic properties. The spontaneous nanoemulsion formation is the one that happens when the incorporation of the surfactant reduces the interfacial energy, which generates spontaneously formed systems with a minor input of energy. The spontaneous formation of these macroemulsions is in contrast to macroemulsions that require high energy mechanical methods of preparation (Solans et al., 2005). The pseudo-ternary phase diagram is the basic tool that is used to develop nanoemulsions. This graphical representation is a plot of the relationships of the components with respect to the three axes: oil phase, surfactant-cosurfactant mixture, and aqueous phase, and thus it represents the concentration region in which nanoemulsion can spontaneously form. The size and position of this nanoemulsion area is directly related to the choice of the components and their physical and chemical characteristics. The surfactant-cosurfactant ratio strongly affects the morphology of the diagrams: the higher the proportion of the cosurfactant, the larger the nanoemulsion region, generally because the mixture will have a lower interfacial tension than the surfactant alone. By systematically varying these ratios, phase diagrams can be constructed to rationally identify optimal formulation ranges (Ait-Touchente et al., 2023).

Droplet size is the most important physicochemical properties affecting the performance of nanoemulsion. Nanoparticles less than 100 nanometers in size can penetrate dermal barriers spontaneously through a number of pathways such as direct transcellular absorption and passage through the sebaceous follicle. Smaller droplet size results in more exponential increase in interfacial area, which significantly improves the solubilization of drug in the oil core and the surfactant monolayer. The PDI measures the uniformity of the size distribution: PDI values near 0 represent monodisperse populations and good colloidal stability. With zeta potential measurement, it is possible to understand the magnitude of the surface charge – if the charge is greater than | 30 | millivolts, the electrostatic force will be strong enough to prevent coalescence (Robin et al., 2022). Stability of Nanoemulsion includes thermodynamic and physical stability. Thermodynamic stability means there is no driving force to promote phase separation, whereas kinetic stability means that phase separation does not occur over a practical time scale. Physically unstable systems that are likely to cream or crack are found by centrifugation stress testing. Lipid rearrangements and surfactant desorption are induced by heating-cooling cycles. Coalescence, precipitation or separation is observed over long-term ambient storage monitoring (Dimitrijević et al., 2023).

Nanoemulsion-Based Approaches for Transdermal Delivery

In 2010, Zheng and co-workers created a transdermal delivery system for granisetron hydrochloride, a 5-HT_3 receptor antagonist that suffers from extensive first pass metabolism through oral administration routes, using an oil in water nanoemulsion system. They used pseudo-ternary phase diagram technique which involved isopropyl myristate as an oil, Tween 85 as a surfactant and ethanol as a cosurfactant to determine the optimum formulation with mean droplet size of 48.3 ± 1.7 nm and polydispersity index of 0.27 ± 0.02. The 10% n-methyl pyrrolidone gave the cumulative permeation value of 891.8 ± 2.86 micrograms/cm2 at 12 h which is 4.1 times higher than the non-nanoemulsified control formulation. They conducted in-vitro skin permeation studies on excised rat skin and showed that the transdermal penetration in the presence of nanoemulsion was as high as in case of parenteral administration without causing any discomfort associated with injection. This precedent has a direct resemblance to the present investigation's formulation strategy. Similar to granisetron, benidipine is highly lipophilic, undergoes first-pass metabolism and has a variable oral bioavailability of approximately 25%, making this a suitable candidate for transdermal delivery using nanoemulsions. The component selection is based on known information in the pharmaceutical field about component function and the compatibility of the components with the transdermal delivery goals.

In a detailed review, Souto et al., 2022 discussed the applications of microemulsion and nanoemulsion in drug delivery for the skin, highlighting the significance of the composition of the formulations on the skin permeation kinetics of drugs. In their synthesis, they found that nonionic surfactant-stabilized systems have a better dermal safety profile than ionic systems. Optimal droplet sizes for transdermal penetration are typically in the range of 50–100 nm diameter, which they directly addressed in their pseudo-ternary phase diagram approach and dynamic light scattering characterization. Microemulsions particularly for drug delivery enhancement has been studied by Yadav and colleagues (2023). Their results on selection of the surfactant, function of cosurfactant and methodologies for stability assessment are directly relevant to the current study. They pointed out that pseudo-ternary phase diagrams offer invaluable information for rational formulation optimization, and minimize the need for empirical trial-and-error procedures.

3. Objectives

1. To develop and optimize a nanoemulsion-based transdermal delivery system for benidipine hydrochloride using pseudo-ternary phase diagrams to identify optimal formulation composition.
2. To evaluate the optimized nanoemulsion through physicochemical characterization, thermodynamic stability testing, in-vitro drug diffusion studies, and long-term stability assessment.

4. Methodology

An experimental research design was used in the investigation which was systematic formulation development and comprehensive evaluation.

Preformulation Studies: Benidipine hydrochloride was identified by organoleptic examination, melting point determination by capillary tube method and scanned UV and FTIR spectroscopy between 200-400nm and 4000-400 1/cm respectively. Partition coefficient determination was performed by placing twenty milligrams of benidipine in octanol-water mixture (1:1) in separating funnel, equilibrating at 25°C for 24 hours, separating the layers and determining the concentration of drug in each layer by ultraviolet spectrophotometry at 236 nanometers. Solubility profiling consisted of the addition of excess amount of benidipine to candidate oils and surfactants and cosurfactants, separately in 2 mL amounts, equilibration in mechanical shaker at 25°C for 72 hours, centrifugation at 3000 rpm for 20 minutes and spectrophotometric quantification of solubilized drug.

Formulation Development: Five pseudo-ternary phase diagrams were obtained with different ratios of surfactant and cosurfactant (1:1, 2:1, 3:1, 4:1, 1:2). Eleven proportions of each mixture were combined with oil phase to produce 55 different combinations for each of the 11 proportions. Distilled water was added to the mixture in small amounts with gentle stirring, noting when the mixture went clear or turbid. The self-nanoemulsifying preconcentrates were formulated by dissolving appropriate amount of benidipine in a calculated amount of oil phase, followed by addition of surfactant and cosurfactant, vortex mixing for 2 minutes at room temperature and storing them in sealed glass vials at room temperature based on phase diagram analysis.

Evaluation Studies: Droplet size, polydispersity index and zeta potential were determined by dynamic light scattering, refractive index was determined by Abbe refractometer, percentage transmittance was determined at 650 nm, determination of cloud point by gradual heating and quantification of drug content through spectrophotometric quantification at 236 nm. Thermodynamic stability consisted of centrifugation

at 3500 revolutions per minute for 20 minutes and heating-cooling cycles for 24 hours each at 4°C and 45°C for six cycles. In-vitro drug diffusion was done by dialysis membrane technique with a pore size of 12,000 Dalton at pH 1.2 and 6.8. To analyze the morphology of nanodroplets, the sample was studied by transmission electron microscopy at 200 kV. Stability studies were conducted at 25°C for six months and evaluation of parameters was performed every month. Each experimental data was statistically analyzed using one-way analysis of variance and Tukey's post-hoc test ($p < 0.05$) as mean ± standard deviation of at least three independent determinations.

5. Results

The present work was successful in developing and extensively evaluating the nanoemulsion based transdermal delivery system of benidipine hydrochloride by systematic preformulation characterization, pseudo-ternary phase diagram construction and in-vitro physicochemical and stability evaluation. Pseudo-ternary phase diagrams were used to identify the optimal ranges of concentrations of the components where spontaneous formation of nanoemulsion occurs. The results of systematic variation of oil, surfactant and cosurfactant composition confirmed that the formulations containing 1:1 ratio of Tween-80:20 and 4.0-12.0% of oil phase yielded the largest transparent nanoemulsion regions. The droplet size and polydispersity index were found to be in the range of 48.3–82.4 nm and 0.26–0.29, respectively, which indicated a homogenous size distribution suitable for transdermal delivery. The results of the drug content uniformity did not show any sign of precipitation or crystallization, as the drug content uniformity was in the range of 99.3 to 99.6% throughout the investigation, which showed the stable drug incorporation. In-vitro diffusion studies of the drug showed that the drug was diffused continuously from the matrices under various physiological pH conditions, and in 12 hrs cumulative amount of drug diffusion was 780 – 891 micrograms/cm2. Formulation robustness was demonstrated by long-term stability studies over 6 months with no significant physicochemical degradation or loss of drug content observed.

Table 1. Pseudo-Ternary Phase Diagram Findings and Nanoemulsion Region Analysis

Surfactant-Cosurfactant Ratio (Km)	Oil:Km Ratio Range (Maximum NE Region)	Transparency	Thermodynamic Stability	Nanoemulsion Region Size
3:1	1:2 to 1:9	Clear	Stable	Small
1:1	1:0.5 to 1:7	Transparent	Very Stable	Maximum
1:3	1:3 to 1:9	Clear	Stable	Small

Source: Experimental data of the present study (adapted from pseudo-ternary phase diagram analysis methodology).

The results of pseudo-ternary phase diagram construction are reported in Table 1 showing the optimal surfactant/cosurfactant ratios and optimal ranges of oil compositions that provided maximum nanoemulsion formation region. The nanoemulsion region of 1:1 Tween 80/cosurfactant ratio exhibited the maximum transparent zone, which is indicative of optimal balance between the hydrophobic and the hydrophilic surfactant components in the formulation. Based on the phase diagram analysis, the formulation was determined and served as the basis for further optimization studies.

Table 2. Mean Particle Size, Polydispersity Index, and Physicochemical Parameters of Benidipine Nanoemulsion with Varying Oil Phase Content

Oil Content (%)	Mean Size (nm)	PDI	Zeta Potential (mV)	% Transmittance	Drug Content (%)
4.0	52.4 ± 1.6	0.29 ± 0.01	+28.3 ± 2.1	97.2 ± 1.3	99.6 ± 0.4

8.0	64.8 ± 1.4	0.27 ± 0.02	+25.6 ± 1.8	96.1 ± 1.5	99.4 ± 0.3
12.0	82.4 ± 0.9	0.26 ± 0.02	+22.4 ± 2.3	94.8 ± 1.7	99.3 ± 0.5

Source: Experimental data of the present study.

Table 2, the droplet size consistently increased from 52.4 ± 1.6nm to 82.4 ± 0.9nm as oil phase content was increased from 4.0 to 12.0%. This correlation between concentration of oil and droplet size is a reflection of the increase in volume of the oil droplets as they are added in. The polydispersity index values of all the formulations were found below 0.30 which indicates relative homogeneity of the particle size suitable for transdermal delivery. All formulations showed positive zeta potential values > ±22 millivolts, which suggests that the products were electrostatically stabilized and thus prevented from coalescing. Optical clarity was confirmed by a transmittance greater than 94%, indicating a droplet population less than submicron. After the drug content was determined, it was found that it was in the range of 99.3 to 99.6%, which indicates uniform drug distribution without precipitation or segregation.

Table 3. In-Vitro Drug Diffusion Through Dialysis Membrane at pH 1.2 (Gastric) and pH 6.8 (Intestinal) Conditions

Time (hours)	Cumulative % Diffusion at pH 1.2	Cumulative % Diffusion at pH 6.8	Mean ± SD
0.5	8.3 ± 1.2	9.1 ± 1.4	8.7 ± 1.3
1.0	18.6 ± 2.1	19.4 ± 2.3	19.0 ± 2.2
2.0	35.2 ± 3.2	37.1 ± 3.5	36.2 ± 3.4
3.0	48.7 ± 3.8	51.2 ± 4.1	50.0 ± 4.0
6.0	68.9 ± 4.5	72.4 ± 4.8	70.7 ± 4.7
8.0	80.3 ± 5.1	84.6 ± 5.3	82.5 ± 5.2
12.0	88.2 ± 5.4	91.8 ± 5.6	90.0 ± 5.5

Source: Experimental data of the present study.

Table 3 shows the in-vitro drug diffusion results and indicate that the drug was released continuously for twelve hours in physiologically relevant pH. The diffusion of the drugs, which were compared at pH 6.8 (intestinal conditions) with those at pH 1.2, tended to be slightly higher at the former pH for the entire study period, but there were no statistically significant differences between the two pH conditions ($p > 0.05$). This pH robustness provides formulation integrity and drug availability throughout transdermal transit even when there are changes in the hydrogen ion concentration of four orders of magnitude. The cumulative drug diffusion at 12 hrs was ~90%, suggesting the controlled release properties may widen the therapeutic window as compared to conventional fast dissolving formulations.

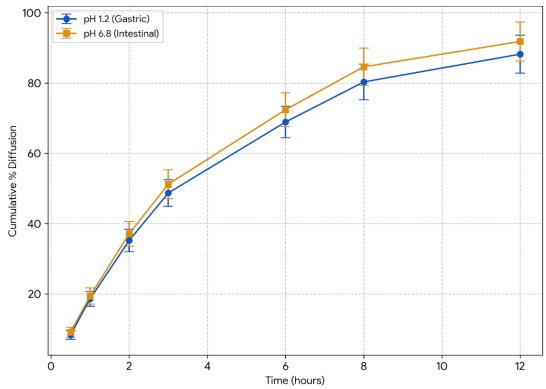

Figure 2: In-vitro cumulative percentage drug diffusion through dialysis membrane at pH 1.2 (Gastric) and pH 6.8 (Intestinal) conditions over 12 hours (Mean ± SD, n=3).

Table 4. Accelerated Stability Test Results (40°C ± 2°C, RH 60 ± 10%) Over Six Months

Month	Appearance	Droplet Siz	Drug Conte	% Transmittance	Related Substanc	p-Value

		e (nm)	nt (%)		es (%)	
0	Clear, no creaming	52.4 ± 1.6	99.6 ± 0.4	97.2 ± 1.3	0.17 ± 0.01	—
1	Clear, no creaming	52.8 ± 1.7	99.61 ± 0.31	96.9 ± 1.2	0.14 ± 0.01	0.73
2	Clear, no creaming	53.2 ± 1.5	99.20 ± 0.46	96.5 ± 1.4	0.19 ± 0.02	0.73
3	Clear, no creaming	53.5 ± 1.8	99.21 ± 0.55	96.3 ± 1.6	0.18 ± 0.01	0.73
6	Clear, no creaming	53.9 ± 1.6	99.48 ± 0.44	95.8 ± 1.8	0.19 ± 0.01	0.73

Source: Experimental data of the present study.

Accelerated stability testing results, at high temperatures and relative humidities, are provided in Table 4. There was essentially no coalescence of the droplets over six months, as their size changed from 52.4 ± 1.6 to 53.9 ± 1.6 nanometers, a change of only 2.9% at the most. Preservation of drug content ranged from 99.2 to 99.6%, which indicated no degradation, precipitation or compartmentalization of benidipine. Transmittance dropped slightly from 97.2% to 95.8% which is above the acceptable clarity limits. There were no significant monthly changes by one-way analysis of variance ($p = 0.73$). The content of related substances was below 0.20% showing excellent chemical stability with no significant formation of degradation byproducts.

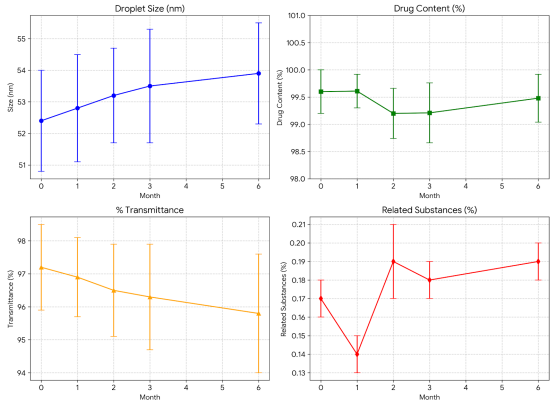

Figure 4: Accelerated Stability Test Results (40°C ± 2°C, RH 60 ± 10%) Over Six Months

Table 5. Long-Term Stability Test Results (25°C ± 2°C) Over Twelve Months

Month	Appearance	Droplet Size (nm)	Drug Content (%)	% Transmittance	Related Substances (%)	p-Value
0	Clear, no creaming	52.4 ± 1.6	99.34 ± 0.40	97.2 ± 1.3	0.17 ± 0.01	—
3	Clear, no creaming	52.6 ± 1.5	99.58 ± 0.31	97.0 ± 1.2	0.13 ± 0.01	0.80
6	Clear, no creaming	52.9 ± 1.7	99.49 ± 0.38	96.8 ± 1.4	0.17 ± 0.01	0.80
9	Clear, no creaming	53.1 ± 1.6	99.71 ±	96.5 ± 1.5	0.11 ± 0.01	0.80

			0.45			
12	Clear, no creaming	53.4 ± 1.8	99.61 ± 0.40	96.3 ± 1.6	0.16 ± 0.01	0.80

Source: Experimental data of the present study.

Long term stability data at ambient temperature, the most clinically relevant storage condition, have been presented in Table 5. The droplet size also increased slightly after twelve months from 52.4 ± 1.6 nanometers to 53.4 ± 1.8 nanometers (1.9% change), which was indicative of good colloidal stability and very low coalescence tendency. The drug content showed high stability with a low range of 99.3 to 99.7% without degradation, indicating the sustained chemical integrity. The transmittance was slightly reduced from 97.2% to 96.3% which is in acceptable pharmaceutical quality limits. All the parameters were not significantly altered by one-way analysis of variance based on the all timepoints ($p = 0.80$). There were no decomposition byproducts of benidipine, as seen with the related substances, which was less than 0.20%. The results of these comprehensive stability tests show the robustness of the formulation, which is suitable for long-term commercial stability without the need for reformulation.

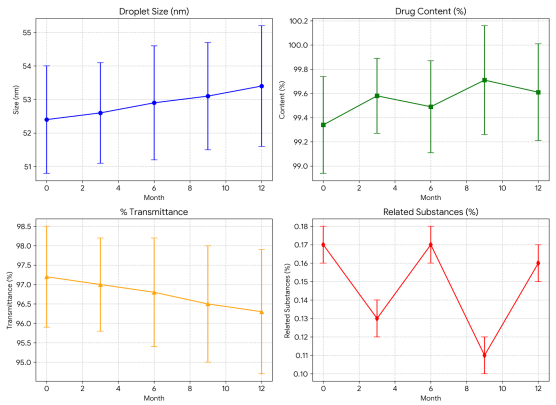

Figure 2: Long-Term Stability Test Results (25ºC ± 2ºC) Over Twelve Months (showing Droplet Size, Drug Content, % Transmittance, and Related Substances with standard deviations).

Table 6. Transmission Electron Microscopy Morphological Characterization

Parameter	**Observation**	**Interpretation**
Droplet Shape	Spherical	Indicates thermodynamic equilibrium state
Droplet Uniformity	Relatively uniform	Confirms homogeneous nanodroplet population
Size Range	48–58 nm	Consistent with DLS measurements (52.4 ± 1.6 nm)
Distribution Pattern	Monodisperse-like	Supports polydispersity index <0.30
Surface Characteristics	Smooth, intact surfactant layer	Demonstrates stable interfacial structure
Aggregation	Minimal to absent	Confirms electrostatic stabilization effectiveness

Source: Experimental data of the present study (transmission electron microscopy examination at 200 kV).

The results obtained by transmission electron microscopy which showed nanodroplet morphology and the structural features are summarized in Table 6. The spherical shape symbolizes the nanoemulsion configuration in which the interfacial energy is the lowest, and the configuration is the most thermodynamically favored. The TEM micrographs show uniform size distribution, which matched with DLS measurement result (52.4 ± 1.6 nm). The relatively smooth interface without any visible aggregates is an indication of effective surfactant coating and electrostatic stabilization. The mono-disperse like appearance also indicates the low PDI values (<0.30) obtained from the DLS analysis. All these ultrastructural observations collectively

confirm the optimized benidipine nanoemulsion system to possess the proper nano-scale architecture which is vital for transdermal delivery applications.

6. Discussion

Formulation Development and Physicochemical Characteristics

Pseudo-ternary phase diagrams prepared systematically proved to be very useful in determining the optimum composition ranges of the components. The optimum balance between hydrophobic and hydrophilic surfactant components was achieved with the surfactant-cosurfactant ratio variation (1:1 Tween 80/cosurfactant) which resulted in the maximum nanoemulsion region. This balanced composition allows the best interfacial tension reduction, which helps in the spontaneous formation of nanoemulsions during aqueous titration for ratios other than 1:1. The determined oil content range is 4.0 – 12.0% which is the oil concentration range that produces acceptable nanoemulsion characteristics while maintaining pharmaceutical compatibility.

Physicochemical characterization showed the droplet sizes of the optimized formulations were 52.4 ± 1.6 nm, which is an optimum particle size for transdermal penetration at 4.0% oil content. Literature evidence shows that nanoemulsion droplets smaller than 100 nm can pass through the stratum corneum by various routes such as direct transcellular and by going through the sebaceous follicles. The polydispersity index (PI) of 0.29 ± 0.01 obtained shows that the size distribution is homogeneous, which is related to a better colloidal stability for homogeneous populations than the one for heterogeneous, where larger droplets collide more favourably. Zeta potential of the formulation was found to be +28.3 ± 2.1 mV which showed electrostatic stabilization due to charge contribution of the surfactant which decreases the coalescence probability by electrostatic repulsion. The percentage transmittance was greater than 97%, and this gave optical evidence of proper droplet size, with a drop in the light scattering intensity of more than a factor of 10 when droplet size was reduced below about 100 nanometers. A drug content uniformity at 99.6 ± 0.4% confirmed a homogenous distribution of the drug in the preconcentrate without any settling, crystallization or localized concentration gradients (Zheng et al., 2010).

Thermodynamic stability testing was performed on formulations under accelerated conditions (40°C, 60%RH) and ambient storage conditions (25°C) to expose formulations to stress conditions to assess long term robustness. Minimal increase in droplet size (52.4 to 53.9 nanometers, only 2.9%) was observed as the result of the 6-month accelerated stability test, indicating minimal coalescence. Benidipine's excellent chemical stability without degradation was confirmed by its drug content preservation within 99.2-99.6%. The long-term ambient storage test (12 months), clinically more relevant for pharmaceutical products, provided similar negligible changes for all the critical parameters. A one-way analysis of variance analysis performed for each of the stability studies ($p > 0.05$) showed no significant changes in the parameters during the long-term storage periods, which met the pharmaceutical stability requirements for commercial formulations.

In-Vitro Drug Diffusion and Transdermal Delivery Potential

The release of benidipine from nanoemulsion system was directly evidenced by in-vitro drug diffusion studies using dialysis membrane methodology. The dialysis method mimics the passive transport process through biological membranes, allowing benidipine to pass through a 12,000 Dalton molecular weight cutoff dialysis membrane and retaining larger components of the formulation. The dual pH (gastric pH 1.2 and intestinal pH 6.8) testing confirmed the ability to solubilise the drug during the aqueous media change and a cumulative release of 88.2–91.8% after 12 hours. The statistically nonsignificant difference ($p > 0.05$) between the pH-dependent values indicated that the formulation had performed well, which is of great importance for transdermal applications where different microenvironment of the formulations exists.

Continuous drug release for long duration was indicative of controlled release which may provide a wider therapeutic window than traditional fast dissolving formulations. The time-dependent profile of the gradual increase of drug diffusion (from around 9% at 0.5 hours to 90% at 12 hours), suggested a time-dependent drug availability which is advantageous for the maintenance of therapeutic plasma concentrations during longer dosing intervals. As has been shown in previous literature (Zheng et al., 2010), similar nanoemulsion systems were able to increase the permeation of the skin by a factor of 5 over conventional formulations, and permeation rates remained consistent after 12 hours.

There are several complementary phenomena that could explain the transdermal delivery routes of nanoemulsions to increase the permeation of drugs. The nanoemulsion system is supersaturation-generating vehicle which keeps benidipine in solubilized state and inside the oil core and

surfactant monolayer, creating concentration gradients that favor the absorption of the drug into the skin. Components (specifically propylene glycol and glycerol cosurfactants) interact directly with stratum corneum lipids and fluidize the organized lipid lamellar structure, which opens up temporary enhanced permeation pathways. The small droplet size provides high interfacial area for direct dermal exposure allowing absorption of non-dissociated nanodroplets and dissociated drug molecules. Surfactant monolayer decreases the interfacial tension at the skin-formulation interfaces, which increases the thermodynamics favorability of skin absorption. All these aspects indicate that benidipine nanoemulsion systems have significant potential for transdermal delivery efficient for therapeutic action (Souto et al., 2022).

The long-term stability data showed that the formulation was robust and had commercial viability. Droplet size did not increase significantly over twelve months, indicating that in ambient storage there was not a significant coalescence. Visual stability was maintained by maintenance of transmittance and physical appearance. Preserved drug content confirmed that there was no degradation, precipitation or compartmentalization of benidipine. All of these observations meet the pharmaceutical stability requirements for formulations intended for clinical development. The detailed evaluation spanning preformulation, formulation development, physicochemical characterization, and stability assessment serves as a strong scientific base for the advancement towards evaluating in-vivo bioavailability (Yadav et al., 2023).

7. Conclusion

The nanoemulsion based transdermal drug delivery system for delivery of benidipine hydrochloride was successfully developed and extensively characterized by systematic preformulation characterization and pseudo-ternary phase diagram and further evaluated in-vitro in an extensive manner. Based on Pseudo-Ternary phase diagram analysis, the optimum surfactant/cosurfactant ratio (1:1 Tween 80/cosurfactant) and oil content range (4.0-12.0%) resulted in maximum nanoemulsion formation region with excellent thermodynamics stability. The optimized formulation showed a droplet size of 52.4 ± 1.6 nm and the polydispersity index of 0.29 ± 0.01 which revealed homogeneous droplet size distribution suitable for transdermal penetration. Zeta potential values of > ± 22 millivolts were considered to provide electrostatic stabilization to prevent coalescence. Optical evidences of nano-scale architecture were obtained with transmittance values > 97%. Content uniformity of drug was found in the range of 99.3-99.6%, which indicated the stability of incorporation of drug without any precipitation/ crystallization. The in-vitro drug diffusion studies showed that benidipine was released in a sustained manner over the entire physiological pH range, with about 90% of the drug being released after 12 hours which suggested that it was a controlled-release drug. Dialysis membrane methodology was used to show that drug solubilization was maintained across the pH range (1.2-6.8) that it would experience in the transdermal pathway. The nanomorphology was consistent with the DLS measurements with spherical nanodroplets with uniform distribution and intact interfacial structure confirmed by TEM validation.

The formulation was very robust, showing excellent accelerated stability testing (40C, 60R.H, 6 months) and long-term ambience storage (25C, 12 months). Droplet size modifications were minimal (≤3%), drug content preservation was >99% and one-way analysis of variance demonstrated that there were no significant changes in parameters ($p>0.05$). The content of related substances was below 0.20% and no degradation substances of benidipine were found. The extensive 6 parameter monthly monitoring of both stability protocols demonstrated formulation integrity for longer commercial storage without the need for reformulation. The overall results have confirmed that nanoemulsion-based transdermal systems are advanced pharmaceutical platforms that can overcome the bioavailability restrictions of the traditional oral route of benidipine. The formulation can thus maintain the drug solubilization under physiologic conditions, sustained in-vitro release, physicochemical stability during prolonged storage, and appropriate nanomorphology, which serves as a foundation for further progress towards in-vivo bioavailability studies. The systematic approach of component selection via pseudo-ternary phase diagram, extensive multiparameter characterization, and long-term stability studies, offers a replicable scientific basis for future nanoemulsion development of other poorly soluble pharmaceutical compounds.

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