

Propranolol nanoemulgel: Preparation, in-vitro and ex-vivo characterization for a potential local hemangioma therapy

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Abstract

A lack of safe and effective topical alternatives to oral propranolol HCl (PHCl) hampers optimal management of infantile hemangioma (IH), particularly in complex cases with severe side effects or treatment failures. This study aimed to develop a nanoemulsion gel (NEG) for topical PHCl delivery. A meticulously formulated nanoemulsion (NE) encapsulated with clove oil, Tween 20, and polyethylene glycol 400 emerged as the standout candidate (NE3) due to its exceptional stability, resilience, and favorable drug loading. NE3 exhibited a remarkable globule size of 14.57 nm, a low polydispersity index (PDI) of 0.282, and a stabilizing zeta potential of -19.89 mV. The subsequent formulation of PHCl-NEG displayed desired rheological and spreadability properties for topical application. Ex-vivo skin retention and permeation studies revealed effective PHCl deposition within the dermal layer with minimal systemic exposure. This promising approach offers a potential alternative to oral PHCl, potentially mitigating severe side effects and improving outcomes in complex IH cases.

Keywords

clove oil, ex-vivo permeation study, infantile hemangioma, nanoemulgel, propranolol-HCl

Introduction

Propranolol HCl (PHCl), a pharmaceutical compound classified as a β -adrenergic receptor antagonist, was first developed for the purpose of managing arrhythmias. Previously, particularly in 2008, it was shown that the oral administration of PHCl to newborns produced significant efficacy in treating infantile hemangioma (IH). Consequently, PHCl has since been established as the primary and sole medicine approved by the FDA for the treatment of IH (Léauté-Labrèze et al. 2008). Up to 10% of the infants are globally diagnosed with IH (McGee et al. 2013; Tiemann and Hein 2020), a common vascular tumor that

requires treatment to prevent profound cosmetic and functional deformities due to skin ulceration and scarring manifests in approximately 15% to 25% of all infants with IH (Laken and Forsythe 2016). Development of IH is linked to certain risk factors such as low birth weight, premature delivery, fertility medicinal products, female gender, white race, and family history (Pahl and McLean 2022).

Administration of PHCl by the oral route has the potential to result in significant complications. Infants should be carefully monitored due to the possibility of systemic exposure to PHCl, which can lead to alterations in sleep patterns, acrocyanosis, and gastrointestinal symptoms. Furthermore, there is a risk of experiencing severe adverse

reactions such as bronchospasm, symptomatic hypotension, hypoglycemia, and bradycardia (Léauté-Labrère et al. 2017). It is important to note that in certain instances, oral PHCl may not provide complete resolution of IH signs and leave residual scars (Lee et al. 2021). In a comprehensive study including 40 pediatric patients, it was observed that a single case exhibited the onset of severe tachycardia within the initial 48-hour period subsequent to the initiation of oral PHCl administration. Therefore, treatment had to be promptly discontinued (Oksiuta et al. 2014).

When it comes to the treatment of superficial hemangiomas, dermal application of PHCl produces far less adverse effects in comparison to the oral administration method. This is due to the advantages of achieving a higher concentration of the medication locally and diminishing its systemic exposure (Al-Haddad et al. 2019). Hence, the compound PHCl, with a LogP value of 1.2 and a small molecular weight of 295.8 g/mol (Al-Majed et al. 2017), has favorable characteristics for potential cutaneous delivery.

In contemporary times, there has been a significant surge in the level of attention and fascination directed towards the field of nanotechnology (Muhammed and Al-Kinani 2023). Nanoscale structures are generated through the utilization of various methods, techniques, and processes of this technology. Advancements in the field of pharmaceuticals have facilitated the progress of nano-carriers, which aim to enhance the targeted transport of drugs to their intended sites of action (Bayda et al. 2020). Nanoemulgel (NEG) is an amalgamated structure of two distinct systems in which a drug-containing nanoemulsion, which is a pharmacokinetically stable colloidal dispersion, usually with a globule size that falls within the range of 10–100 nm (Srivastava et al. 2016), is incorporated into a hydrogel base.

Topical formulations of PHCl have been the subject of several investigations that have demonstrated their safety and effectiveness to some extent, but some cases of partial resolution have been reported in the context of newborns with IH. To name several of these pharmaceutical hydrophilic preparations, a gel containing 3% PHCl (Wang et al. 2012), an ointment containing 1% PHCl (Wahab et al. 2017), and a cream containing 1%, 3%, and 5% PHCl (Kashiwagura et al. 2022). The work conducted by He et al. (2021) investigated the use of PHCl using microneedles for cutaneous delivery.

To overcome the challenges of oral PHCl in complex IH, including significant side effects and treatment resistance, this study explores a topical nanoemulsion gel (NEG) containing PHCl. This novel approach seeks to target affected skin regions directly, potentially minimizing systemic exposure and improving outcomes in cases where complete resolution remains elusive.

Materials and methods

Propranolol-HCl, Tween 20, glycerin, and Carbopol (934) were procured from Awamedica Pharmaceuticals Factory

(Erbil, Iraq). PEG400 was obtained from Sigma-Aldrich Co., (St Louis, MO, USA). Clove essential oil and triethanolamine were supplied by Alpha Chemika Co., Ltd. and Thomas Baker Pvt., Ltd. (Mumbai, India) respectively. Double distilled deionized water was produced by a Milli-Q purification instrument (Baghdad, Iraq).

All additional reagents utilized in the experiments were of analytical grade. The full-thickness skin samples of male “Wister Albino” rats, with an average weight of 140 ± 10 g, were acquired from the SLAC Laboratory Animal at the Pharmacy College in Baghdad, Iraq. Ex-vivo permeation investigations and drug deposition experiments involving rat skin were conducted with the consent of the Institutional Animal Care and Use Committee at the Pharmacy College, Baghdad University.

Development and formulation optimization

For the development of drug-loaded NEs, the evaluation and optimization of formulation components relied on how well the payload dissolved in the saturation solubility study and how the phases behaved between the formulation excipients. The saturated solubility of PHCl was determined in various oils, surfactants, and co-surfactants using the shake-flask method. In this approach, an excess amount of PHCl was added to 5 g of each individual component (Altamimi et al. 2019). The oil phase was evaluated by assessing its solubilization capacity for PHCl, while surfactant selection was based on their respective abilities to emulsify the oil phase and their maximum solubilities in PHCl (Sumaya Shaimaa 2021). The selection of co-surfactants was based on their greatest nanoemulsifying areas, which were determined by generating pseudo-ternary diagrams along with the surfactant and oil phases by adopting the aqueous titration method (Hussein and Rajab 2018). Oil phase was blended with a mixture containing a surfactant and co-surfactant at various weight ratios in glass vials of varying compositions, ranging 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9. The structure was homogenized by vortexing it for 5 min. Next, deionized distilled water (DI DW) was titrated with each composition under constant stirring, and the quantity of water added was recorded until the development of a milky or turbid endpoint (Tang et al. 2019).

Nanoemulsion preparation and assessment

By using a vortex mixer and the optimal component ratio determined from pseudo-ternary phase diagrams, PHCl-loaded NEs were generated by the low-energy emulsification approach (Dahash and Rajab 2020). Following the achievement of consistent miscibility between the structure constituents, the entire system underwent vortexing for an interval of 5 minutes. The aqueous phase was incrementally introduced into the mixture utilizing a magnetic stirrer operating at a speed of 500 rpm.

This process aimed to attain a transparent dispersion of colloidal globules in a nanoemulsion (NE) structure. Subsequently, the NE underwent ultrasonication for an extra duration of 5 min (Taher et al. 2022). Once generated, the nanoemulsion structures were stored in photosafe, hermetically sealed glass containers at a controlled temperature for subsequent characterization experiments.

In-vitro drug diffusion study

The study assessed the in-vitro diffusion rates of PHCl in NE structures using a Franz diffusion cell system. The receptor section of the cell has a volume of 12 mL and an effective diffusion area of (1.767 cm²). This section was separated from the donor section by a synthetic semipermeable membrane with a molecular weight cut off of 8,000–14,000 D, which served as a barrier for diffusion. A sample of the prepared PHCl-loaded NE was placed in the donor compartment of the Franz diffusion cell, which contained acetate buffer saline at a pH of 5.6. The stirring rate was fixed at 50 rpm, and the temperature was maintained at 37 ± 0.5 °C (Vartak et al. 2020). The receptor compartment was sampled at regular intervals and refilled with fresh medium (1 mL aliquots). PHCl concentrations were determined by UV-spectroscopy (Nashat and Al-Kinani 2023).

Nanoemulgel preparation and assessment

For topical application, nanoemulgel containing the optimum NE structure were produced with Carbopol 934 at a concentration of 1% w/w. The measured quantities of the selected gelling polymer were dissolved in deionized distilled water, and the mixture was allowed to sit overnight for the purpose of achieving homogeneous swelling (Daood et al. 2019). It was decided to add glycerin as a humectant to the dispersion structure in order to give a silky and calming sensation to the generated nanoemulgel. By gradually adding triethanolamine to the dispersion structure, the preparation underwent cross-linking, and its pH was adjusted to 5.6, resulting in instantaneous transformation into a hydrogel system (Ahmad et al. 2019). Ultimately, the optimum NE structure was uniformly integrated into a blank gel in order to produce PHCl-loaded nanoemulgel (PHCl-NEG). The following characteristics were used to evaluate the PHCl-NEG in terms of its rheology, spreadability, acidity, extrudability, and drug content uniformity:

Rheology behaviors

A rotational viscometer (Myer rotary viscometer VR 3000, spindle no.7; Vendrell Instruments Ltd., Barcelona, Spain) was used to examine the PHCl-NEGs' rheological behaviors at 25 ± 1.0 °C. In order to characterize the PHCl-NEGs' shear-stress profiles and thixotropic behaviors, we subjected them to a series of simulated topical administrations at shear rates ranging from 10 to 200 s⁻¹ over the course of eight stages, with 30 s of equilibration time between each step (Md et al. 2020).

Spreadability

The spreadability of the PHCl-NEG and a commercially available emulgel product (Voltaren emulgel) was assessed by inserting a precisely measured quantity (1.0 g) of the sample between two glass plates having a surface area of 20 × 20 cm² and a width of 6 mm for a duration of 1 min. The upper plate was used as a normal-weight compressor of 240.0 g. The measurement of spreadability was stated as a diameter function relating the spreading area to the applied weight (Chen et al. 2016).

pH analysis

A total of 50 mL of DI DW was added to a known weight of the prepared nanoemulgel (5.0 g). Using a digital pH meter (Hanna Instruments HI 98107 Bucharest, Romania), the PHCl-NEG system was diluted to a concentration of 10% w/v, stirred thoroughly for 15 min, and its pH was measured (Daood et al. 2019).

Drug content uniformity

A quantity of 1.0 g of the formulations was collected from random sections of the prepared PHCl-NEG. The samples were diluted using methanol and subjected to sonication for 15 min. The extracts underwent centrifugation at a speed of 3000 rpm for a duration of 15 min. Subsequently, the resulting supernatants were subjected to filtration using a syringe filter made of a 0.45 µm pore diameter membrane. The concentration of PHCl in each extract was quantified utilizing a UV spectrophotometer (Algahtani and Ahmad 2020).

Ex-vivo skin deposition and permeation investigation

The evaluation of the ex-vivo skin permeation of PHCl from the NEG system was conducted utilizing the Franz diffusion cell. A specimen of shaved and dissected posterior skin from Wister Albino rats was affixed to the two compartments of the cell, as displayed in Appendix 1: fig. A1. The PHCl-NEG sample (300 mg) was introduced into the donor compartment, whereas the receptor compartment was filled with a medium consisting of acetate-buffered saline at a pH of 5.6. The entire setup was then kept at a temperature of 37 °C. Samples were obtained at various time points of 15, 30, 45, 60, 90, 120, and 150 min and substituted with an equivalent volume of receptor media. The samples were examined employing a UV spectrophotometer with a maximum wavelength (λ_{max}) of 290 nm. The process of measuring skin permeation was subsequently replicated for the PHCl gel of 1% w/w. The purpose of this procedure was to compare the cutaneous penetration of PHCl versus that of the newly developed NEG systems.

The permeation profile was determined by plotting the cumulative amount (Q, in µg/cm²) of PHCl that penetrated per unit area across the skin as a function of time. By conducting a plot, various parameters can be derived. These parameters encompass:

The rate of permeation, denoted as J_{ss} , is measured in $\mu\text{g}/\text{cm}^2/\text{min}$. It was determined by calculating the slope of the linear section of the regression line, as shown in the equation below:

$$J_{ss} = dQ/dt$$

The permeability coefficient, denoted as K_p and measured in cm/min , was determined using the following equation:

$$K_p = J_{ss}/C_0$$

The variable C_0 represents the initial drug concentration in the donor compartment.

The enhancement ratio, denoted by E_r , was calculated by dividing the flux of PHCl-NEG by the flux of PHCl-gel, as shown in the following equation:

$$E_r = J_{ss} \text{ of PHCl-NEG} / J_{ss} \text{ of PHCl gel}$$

Simultaneously, investigations were conducted to analyze the skin deposition of both PHCl-NEG and PHCl gel (Tayah and Eid 2023). The experimental procedure involved thoroughly washing the skin portion with de-ionized distilled water, followed by a skin permeation analysis. Subsequently, the skin samples were soaked in methanol for 24 h after thorough homogenization by a Homogenizer HG-150 (Witeg Labortechnik, Wertheim, Germany) at 5000 rpm for 5 min, ultrasonicated for 15 min on the day after, and the resulting supernatant was filtered through a $0.45 \mu\text{m}$ filter syringe. The drug deposit was then measured using spectrophotometry, providing valuable insights into the enhancement ratio of the PHCl-NEG system that was generated (Algahtani and Ahmad 2020).

Stability assessment

The stability of the developed PHCl-NEG system was assessed by storing it at ambient temperature ($25 \pm 2 \text{ }^\circ\text{C}$) and in the refrigerator ($4 \pm 2 \text{ }^\circ\text{C}$) for a period of three months. The specimens were inserted into photoprotective glass tubes and retrieved afterwards at regular intervals (0, 1st, 2nd, and 3rd month) in order to examine the alterations in their physical characteristics, pH levels, rheological properties, and drug content percentage (Chen et al. 2016). Stability studies were meticulously conducted at controlled temperatures, and the nanoemulsified gel (NEG) was stored in airtight containers to prevent content loss.

Statistical analysis

The experimental results are presented as the average of three samples along with their standard deviations. The data were analyzed using one-way ANOVA (Tukey's post-hoc test). Differences were considered statistically significant when $p < 0.05$.

Results and discussion

Formulation assessment

The solubility of PHCl, a solid substance with a white crystalline structure, was determined at room temperature. It was observed that PHCl had a significantly higher solubility in clove oil compared to that in other oils which is clearly shown in Fig. 1.

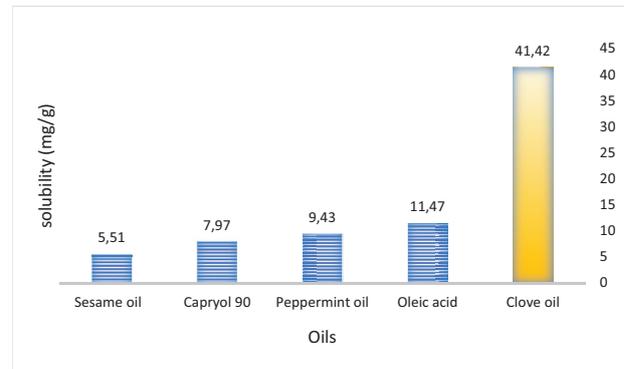


Figure 1. Histogram of PHCl solubility in different oils.

Clove oil is a potent essential oil derived from the distillation process of the flower buds, stems, and foliage of clove trees (*Eugenia aromatica* or *caryophyllata*, Fam. Myrtaceae) (Mylonas et al. 2005). Eugenol, the primary extracted component of clove oil (82–88% of its composition), is a pale-yellow oil that imparts the distinctive odor of cloves. It possesses specific properties, such as antipyretic, analgesic, anti-inflammatory, and anesthetic effects (Mohammadi and Başaran 2017). Moreover, it exhibits antioxidant (D'Avila Farias et al. 2014), and antibacterial properties (Manganyi et al. 2015), which are beneficial for the treatment of infantile hemangiomas. Eugenol is widely used in various industries, such as pharmaceuticals, cosmetics, dentistry, food processing, and agriculture, owing to its non-toxic nature, absence of adverse effects, and lack of residual metabolites (Alam et al. 2017).

PHCl had favorable solubility when combined with Tween 20 as a surfactant and PEG 400 as a co-surfactant (Table 1). The achievement of maximal drug solubility in the oil phase is a desirable characteristic in the development of an NE due to its ability to facilitate the incorporation of a high drug dose inside the formulation (Taher and Hussein 2015).

The elevated solubility of PHCl in clove oil as an oil phase can be attributed to the demonstrated strong hydrogen bonding capacity of clove oil. This characteristic

Table 1. Solubility of PHCl in different oils, surfactants, and co-surfactants.

Constituents	Solubility (mg/g)
Tween 20	13.12 +/- 1.05
Cremaflor EL	9.82 +/- 0.98
PEG 400	46.06 +/- 1.93
Transcutol P	27.71 +/- 1.15

potentially elucidates the rationale behind its pronounced solubility for PHCl. It is possible that clove oil's solubility is due, in part, to the presence of eugenol. The structure of PHCl is depicted in Fig. 2.

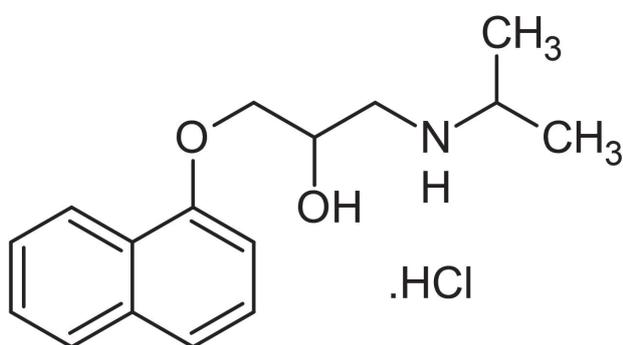


Figure 2. Chemical structure of PHCl.

The hydrophilic-lipophilic balance (HLB) value of the surfactant structure is critical for monitoring the emulsification of the aqueous and oil phases and developing a nanoemulsion. Non-ionic surfactants with high HLB values, such as Tween 20 at 16.7, improve globule stability, making them better for drug delivery systems (Eskandani et al. 2013). Co-surfactants were employed in conjunction with surfactants to confer pliability to the surfactant monolayer surrounding the nanoemulsions. In addition, co-surfactants are essential for mitigating repulsive forces and enhancing the mobility of the aqueous and oil phases.

A study of phase behavior was conducted to examine the effects of different component ratios in relation to the mixture of the surfactant and co-surfactant in the formulation on the development of NEs. Using a pseudo-ternary phase diagram, we can see how the mass proportion of surfactant to co-surfactant (Km) is related to the phase behavior of an NE (Md et al. 2020).

The structure experienced changes in its optical properties as it transitioned from a transparent state to a translucent state, and eventually to an opaque state. These transitions were the result of reorganization occurring within the constituents present in the non-equilibrium phase, leading to alterations in the system's light-scattering characteristics. The phase behaviors of the preformulated NE comprising these components at different ratios are presented in Table 2.

Table 2. Behavior investigation in the preformulation phase for developing of PHCl-NEs.

Oil phase	Drug load	Km phase	Km ratio	Deduction
Clove Oil	Blank	Tween 20 and PEG 400	1:2	Translucent emulsion >100 nm
Clove Oil	Blank	Tween 20 and PEG 400	1:1	Transparent nanoemulsion <100 nm
Clove Oil	Blank	Tween 20 and PEG 400	2:1	Transparent nanoemulsion <50 nm
Clove Oil	Blank	Tween 20 and PEG 400	3:1	Transparent nanoemulsion <20 nm

The NE region was utilized for the assessment of Km, and a positive correlation was seen between the size of the NE region and the efficiency of nanoemulsification in the overall structure. Many formulae are feasible for the pseudo-ternary phase diagram's NE region. A transparent dispersion NE system and globule size of less than 100 nm can be achieved with up to 10% oil phase w/w. Consequently, by aqueous titration, the generally recognized as safe (GRAS) grade Km phase (Tween 20 and PEG 400) and the oil phase (clove oil) were utilized to investigate phase diagrams. Clove oil is thought to be cytotoxic, yet the phenoxyl radical produced by eugenol is much more stable and therefore, far less reactive than reactive oxygen species (ROS), which provide protection (Tisserand and Young 2013).

Fig. 3 depicts the effect of Km on the size of the NE region and phase behavior, as shown in the pseudo-ternary phase diagram.

Nanoemulsion preparation components

From the results obtained from the saturation solubility study, PHCl was dissolved in clove oil as the oil phase, and then the Km phase (Tween 20 and PEG 400) was blended using a vortex mixer. Transparent dispersion NE structures with a drug payload were generated as the aqueous phase was then titrated promptly, and the resulting solution was homogenized by ultrasonication.

Effect of Km on nanoemulsification

The present experiment aimed to evaluate the impact of varying the surfactant-co-surfactant mass ratio (Km) on the globule size and polydispersity index (PDI) of PHCl-loaded nanoemulsions (NEs). This investigation was conducted by preparing a series of NEs with fixed composition and altering the Km ratios (1:1, 2:1, and 3:1) of the selected optimal components. The findings of the study revealed that an increase in surfactant concentration, in relation to the co-surfactant content, had a substantial impact and was inversely correlated with the average globule size of the resulting nanoemulsion. Furthermore, it was observed that this had also had an inverse impact on the PDI of the NEs structures. The PDI followed the order of NE1 > NE2 > NE3, as depicted in Fig. 4.

These results indicated that the formulations characterized by a greater Km ratio exhibited better-performing PHCl-NE structures. The observed phenomenon can be attributed to the enhanced solubilization and improved hydrophilicity of PHCl, which are facilitated by greater amounts of Tween 20 in the Km ratio (Chen et al. 2017).

Evaluation of PHCl nanoemulsions

The prepared NE formulations utilized in the investigation of the phase diagram were stressed by thermodynamic stability assessment, encompassing procedures such as heating-cooling cycles, centrifugation tests, and freeze-thaw cycles.

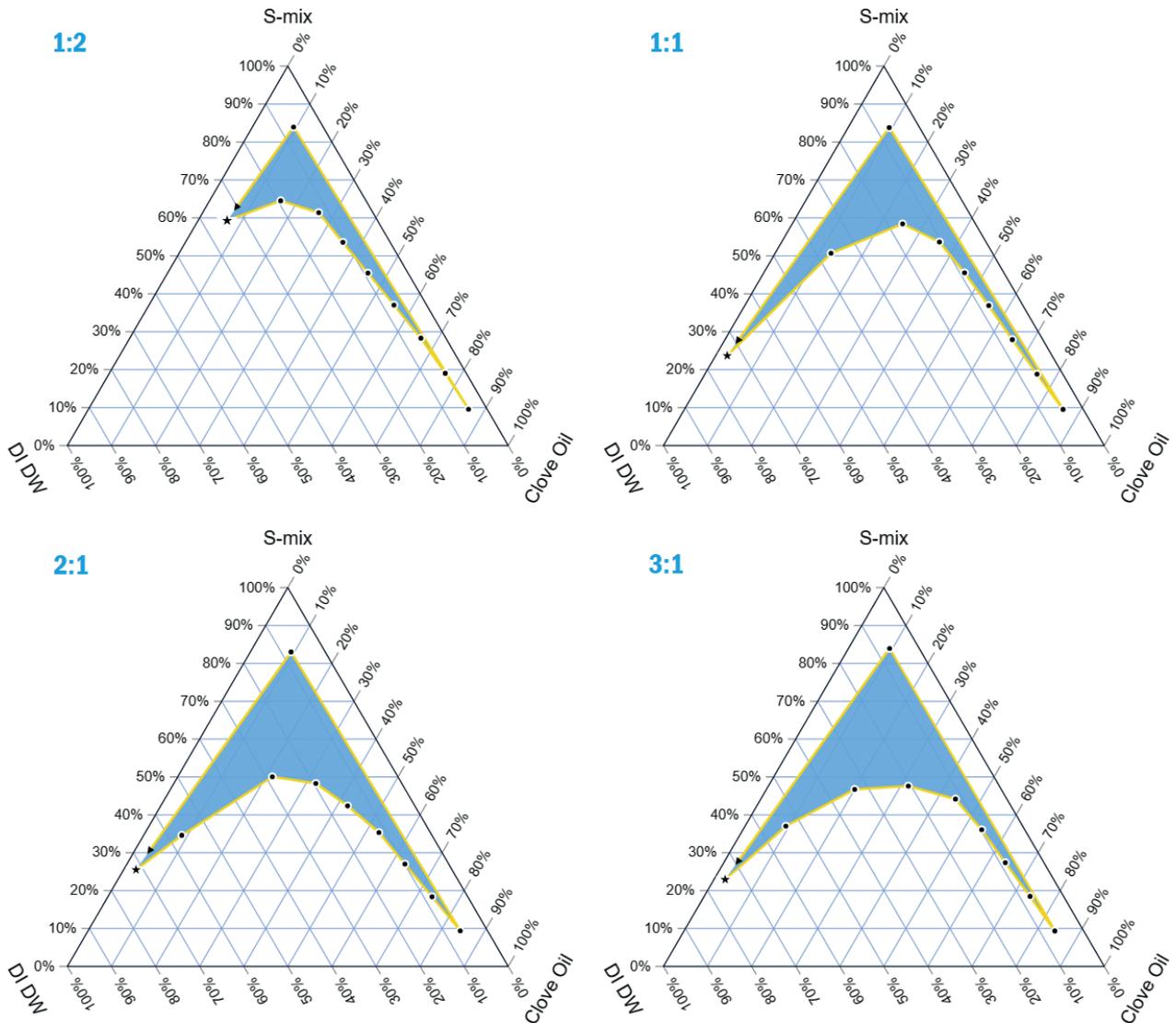


Figure 3. Pseudo-ternary phase diagrams of PHCl-NEs at various Km ratios.

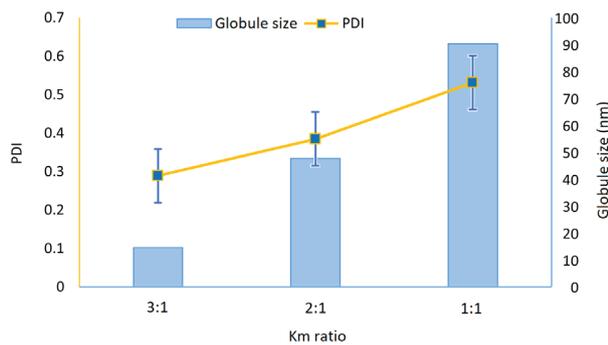


Figure 4. The impact of the Km ratio on globule size and PDI of the generated NEs.

All formulations (Table 3) that were subjected to testing, namely NE1–NE3, exhibited no discernible signs of nanoemulsion instability, including creaming, cracking, or coalescence.

Furthermore, all the formulations successfully completed the stress tests. The percentage of light transmittance, %T, for the generated NE structures (NE1–NE3)

Table 3. Composition of PHCl-NEs.

Formulation	Nanoemulsion Composition (%w/w)			
	Oil	Tween20	PEG 400	DI DW
NE1	10.0	30.0	30.0	30.0
NE2	10.0	40.0	20.0	30.0
NE3	10.0	45.0	15.0	30.0

was found to be greater than 98%, suggesting that the structures under investigation were in a finely dispersed state (Table 4). Additionally, the tested formulations displayed perfect transparency, which indicated that the globule sizes were in the nano-range based on negligible light scattering.

The electrical conductivity of the produced NEs was tested, and the observed pattern in conductivity, NE3 > NE2 > NE1, indicated that an increase in the surfactant ratio resulted in higher conductivity levels. The successful synthesis of oil-in-water (o/w) nanoemulsions (Nashat and Al-Kinani 2023) was confirmed through the test results presented in Table 4. The aqueous dilution durability of the NE structures was evaluated, and it was discovered

Table 4. Evaluation of PHCl-NEs.

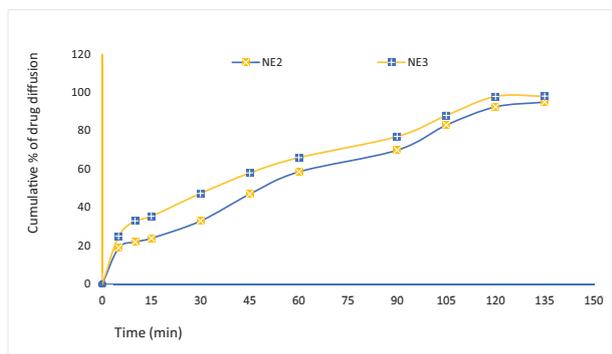
Formulation	Conductivity (σ) $\mu\text{S}/\text{cm}$	%T	% Drug content	Mean globule size (nm)	PDI	Dilutability
NE1	68.0	98.18 \pm 0.93	98.43 \pm 0.61	90.06 \pm 1.16	0.531 \pm 0.13	Opaque $\times\times$
NE2	75.0	99.06 \pm 0.52	98.79 \pm 0.36	47.78 \pm 0.61	0.385 \pm 0.06	Clear \surd
NE3	81.0	99.33 \pm 0.49	99.02 \pm 0.42	14.57 \pm 0.25	0.289 \pm 0.008	Very clear $\surd\surd$

that NE3 and NE2 exhibited improved capacities as the concentration of the surfactant increased. However, NE1 did not pass the test, as evidenced by the appearance of turbidity (Appendix 1: Fig. A2).

In the context of topical applications, it is desirable for the globular size of NEs to be smaller than 50 nm, accompanied by a PDI value of less than 0.30, and effective stabilization with a zeta potential ζ that is as modest as -30 mV (Honary and Zahir 2013). This characteristic facilitates a greater surface area, enabling the enhanced penetration of a larger quantity of payload into the desired target. In light of this, the NE2 and NE3 formulations were chosen for utilization in the in-vitro investigation of drug diffusion.

Investigation of in-vitro drug diffusion

The observations of in-vitro diffusion of PHCl from the NEs (NE2 and NE3) indicated that there was a complete diffusion of PHCl from both NE2 with 96% and NE3 with 99% after a duration of 120 min (Fig. 5).

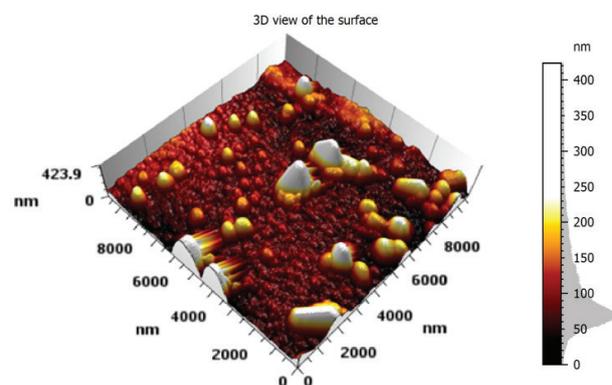
**Figure 5.** In-vitro drug diffusion profiles of PHCl-NEs (NE2 and NE3).

The NE3 formulation structure exhibited the highest level of PHCl diffusion, likely because of its smaller average globule size in comparison to the other NE preparation (NE2). Based on the obtained findings, NE3 was identified as the most suitable NE for incorporation into an NEG framework. The mean globule size and PDI of the varying NEs preparations (NE1–NE3) as provided by Table 4 are illustrated in Suppl. material 1: figs S1–S3.

Morphology rationalization with AFM

The dimensions and structure of PHCl NE3 were verified using high-resolution imaging with the atomic force microscopy (AFM) technique. The results substantiated that the NE globules exhibited a spherical morphology and had dimensions within the nanoscale range (Ho et al.

2022). This was confirmed by analyzing the globule size surface topography through 3D visualization, as depicted in Fig. 6.

**Figure 6.** Surface 3D view of PHCl-NE3 by AFM.

Nanoemulgel assessment and evaluation

The systemic NE3 was included in the prepared hydrogel network of Carbopol 934 (1.0% w/w) resulting in the generation of a PHCl-NEG system that demonstrated a transparent, consistent, viscous gel appropriate for topical application. It had an elegant appearance with a transparent golden hue devoid of any gritty particles or aggregate that could be felt by the thumb [Appendix 1: Fig. A3A]. The rheological characteristics of NEG were assessed. The NEG system demonstrated non-Newtonian pseudoplastic flow behavior when the transition from gel-to-solution began, displaying shear-thinning characteristics and a thixotropic response upon the application of shear stresses [Appendix 1: Fig. A3B]. The gel recovery process was observed to commence, as evidenced by the drop in shear stresses as the shear rate reversal from (200–10 s^{-1}). For both shear rate directions, the measured viscosity data was convergent, ruling out the possibility of rheopexy (dilatant) behavior.

When it comes to the topical distribution of medications, the pseudoplastic behavior of gel formulations is both practical and preferable (Lee, et al. 2009). The rheological measurement data for the newly finalized NEG can be observed in Fig. 7.

When constructing a semi-solid pharmaceutical formulation that is meant for cutaneous application, adequate spreadability helps ensure uniform distribution of topical gels; moreover, this aspect is seen as a critical determinant of patient adherence to treatment (Chen et al. 2016). In this test, the spreadability data was designed to be collected with the minimum application of shear force, with the affected area of infantile hemangioma in mind.

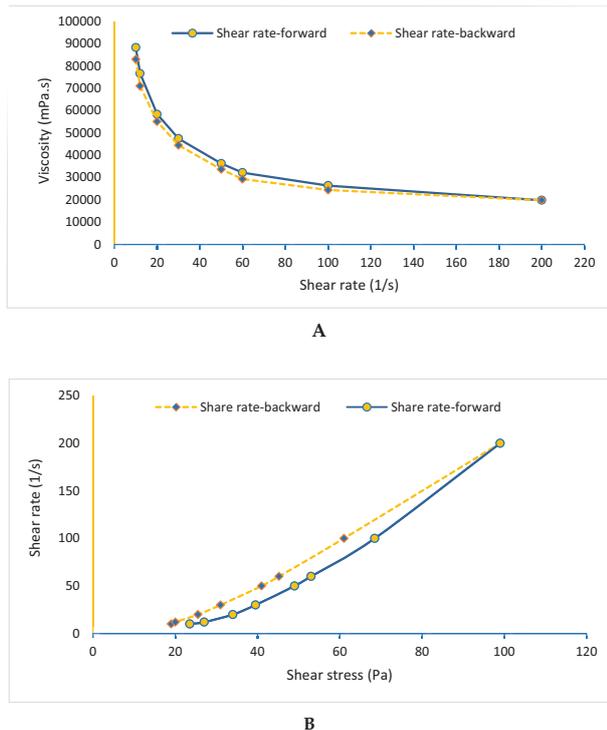


Figure 7. The rheological relationships of PHCl-loaded nanoemulgel (PHCl-NEG) between: **A.** Viscosity and shear rate; **B.** Shear rate and shear stress.

The measured spreading values of NEG system after 1 min are depicted in Fig. 8. These values are given in terms of diameter. According to the study's findings, PHCl-NEG's spreadability was on par with that of the commercial product utilized as a reference.

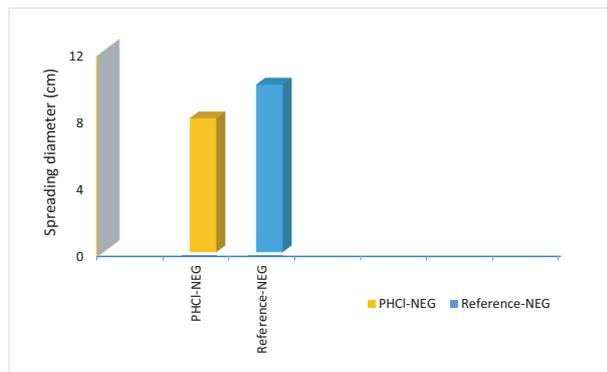


Figure 8. Spreadability values for the PHCl-NEG compared to the marketed product.

The pH discrepancy between the pH of the generated PHCl-NEG and the pH of skin may lead to skin irritation. Topical formulations should preferably have a pH within the range of the skin's pH to avoid disrupting the skin's acid layer. The pH value of the generated PHCl-NEG was determined to be 5.66 ± 0.04 , showing a close resemblance to the pH level of the skin acid shield. Furthermore, the PHCl-NEG exhibited favorable extrudability characteristics, allowing for convenient dispensing by end-users. The generated NEG underwent uniformity and drug content

analyses, which revealed a consistent distribution of PHCl throughout the entire system. The uniformity percentage of PHCl in the NEG was evaluated as 99.54%, which was determined based on the drug content of $98.90 \pm 0.46\%$.

Skin deposition and permeation outcomes

An ex-vivo drug deposition study was conducted to compare the parameters of PHCl-NEG and PHCl gel. The results in Table 5 show that the cumulative permeation and skin retention of PHCl in skin treated with PHCl-NEG was statistically significant in comparison with those treated with solely PHCl gel ($p \leq 0.05$).

Table 5. Evaluations of the gel systems from skin deposition and permeation investigations.

Variables	PHCl-NEG	PHCl gel
Drug deposited in skin ($\mu\text{g}/\text{cm}^2$)	583.38 ± 15.07	198.02 ± 12.24
Cumulative amount of drug permeated (μg)	298.67 ± 14.87	166.54 ± 7.69
Jss ($\mu\text{g}/\text{cm}^2 \text{ min}$)	4.64 ± 0.36	1.76 ± 0.28
Permeability coefficient ($K_p \times 10^{-2}$)	4.12 ± 0.003	1.56 ± 0.002
Enhancement ratio (ER)	2.65 ± 0.23	

By analyzing the PHCl-NEG system and conventional PHCl gel, the enhancement ratio (ER) for the permeation of PHCl diffused from the PHCl-NEG system was 2.65 ± 0.23 . The quantity of PHCl that was deposited in the skin by PHCl-NEG was found to be much larger ($583.38 \pm 15.07 \mu\text{g}/\text{cm}^2$) compared to the amount deposited by the PHCl gel ($198.02 \pm 12.24 \mu\text{g}/\text{cm}^2$), with a more than triple increment. Likewise, dermal drug flux (Jss) of PHCl from PHCl-NEG system was found to be significantly higher (4.64 ± 0.36) compared to the Jss obtained from PHCl gel (1.76 ± 0.28), indicating a more than 200% increase in drug flux as displayed in Fig. 9.

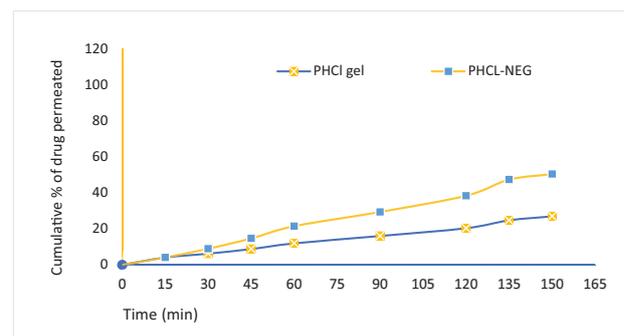


Figure 9. Comparative ex-vivo permeation analysis shows a statistically significant difference between PHCl-NEG and PHCl gel.

Physical stability study findings

After three months of storage at surrounding temperatures ($25 \pm 2^\circ\text{C}$ and $4 \pm 2^\circ\text{C}$), the PHCl-NEG was found to exhibit excellent physical stability. Indicated by Table 6, the attractiveness, pH, rheology, and drug content all remained intact, indicating a remarkable degree of resilience ($p > 0.05$).

Table 6. Stability study parameters results of PHCl-NEG.

Storage period	Rheological behavior	PHCl content (%)	Phase separation	pH	Visual appeal
1 st month	Pseudoplastic	98.36 ± 1.19	Nil	5.66 ± 0.03	Transparent/Golden
2 nd month	Pseudoplastic	98.31 ± 0.87	Nil	5.64 ± 0.04	Transparent/Golden
3 rd month	Pseudoplastic	98.25 ± 1.26	Nil	5.62 ± 0.02	Transparent/Golden

Conclusions

PHCl was successfully encapsulated in a colloidal dispersion of clove oil, Tween 20, and PEG 400. The characterization approaches indicated that NE3 had superior performance and durability, making it the optimum choice for forming a PHCl-NEG structure, which possesses exceptional stability, spreadability, and desirable rheological behaviors. The ex-vivo skin deposition and permeation assays demonstrated that the nanoemulgel effectively retained the PHCl within the skin layers, the specific location where the medication exerts its therapeutic effects. In addition, the nanoemulgel facilitated the cutaneous permeation of PHCl, albeit in limited quantities.

In the end, the outcomes of our investigation demonstrate that the utilization of the PHCl-NEG system through

topical application presents a potentially enhanced substitute for the oral administration of PHCl in instances of complicated IH characterized by severe systemic adverse events and/or failed treatment. This alternative may be particularly beneficial in cases in which complete resolution is not attained leading to permanent cosmetic morbidity. Nevertheless, the research emphasizes the necessity for further clinical and cytotoxicity trials in order to rigorously delve more into the efficacy and safety of this innovative therapeutic strategy.

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Appendix 1



Figure A1. Specimen of skin from Wistar Albino rat.



Figure A2. Photographic pictures displaying the dilutability of PHCl-NEs.

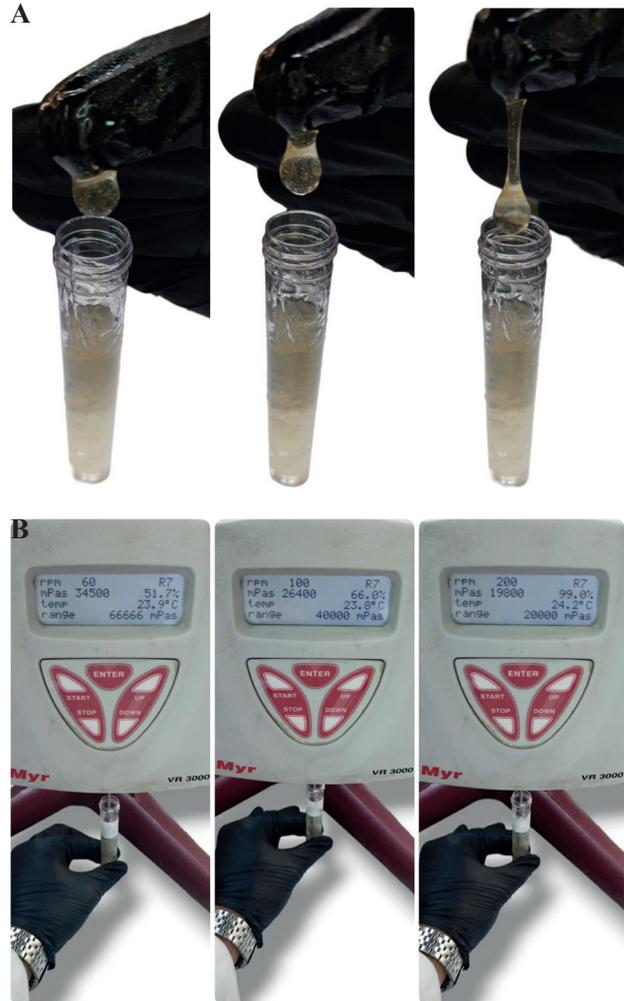


Figure A3. Photographic pictures displaying PHCl-NEG: **A.** Consistency and physical appearance; **B.** Rheological behavior measurement.

Supplementary material 1

Supplementary information

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Data type: docx

Explanation note: The following supporting information regarding PHCI-NE structures includes: **fig. S1**. Globule size (91.06 Nm with PDI 0.531) of NE1; **fig. S2**. Globule size (47.70 Nm with PDI 0.352) of NE2; **fig. S3**. Globule size (14.87 Nm with PDI 0.282) of NE3.

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