Topical Propranolol Hydrochloride Nanoemulsion: A Promising Approach
Drug Delivery for Infantile Hemangiomas

Taif Mohanad Abdullah¹,¹ and Khalid Kadhem Al-Kinani²

¹Ministry of Health and Environment, Baghdad, Iraq.
²Department of Pharmaceutics, College of Pharmacy, University of Baghdad, Baghdad, Iraq.

Abstract
Infantile hemangioma (IH) is a prevalent vascular tumor that affects up to 10% of infants worldwide. If left untreated, IH can lead to a permanent notable physical disfigurement due to skin lesions’ ulceration and scarring. Oral propranolol hydrochloride (PHCI) is the only (FDA) approved, first-line treatment for infantile hemangioma (IH), but systemic exposure to propranolol HCl can cause serious adverse reactions like: bronchospasm, symptomatic hypotension, hypoglycemia, and bradyarrhythmia. To reduce the risk of adverse effects, topical delivery is preferred due to its ability to provide high local drug concentration and fewer systematic side effects. Therefore, PHCI with LogP equal to (1.2) and molecular weight of (295.8 g/mol), is a good candidate to be dermally delivered and penetrate the stratum corneum (SC) barrier. Nanoemulsion (NE) was the chosen pharmaceutical technique to be utilized for topical delivery. NEs have evolved as a robust carrier for the delivery of a diverse spectrum of hydrophilic and hydrophobic drugs. PHCI was formulated as a NE employing clove oil as an oil phase, Tween 20 as surfactant and either (Poly ethylene glycol 400 or Propylene glycol) as a co-surfactant and (DI DW) as aqueous phase. Ten formulas were prepared, and different tests were accomplished to characterize the NEs, such as electrical conductivity, globule size, polydispersity index, percent of light transmittance (T%), dilution test, pH, zeta potential, drug content, viscosity and in-vitro drug leakage. Results of analysis revealed that more than one PHCI loaded nanoemulsions formula like (F5) and (F9), with (clove oil 10%) (S-mix 60%) and (DI DW 30%) could be optimized for further dosage form development, since both of them had a globule size of (<50 nm), low PDI (>0.3), light transmittance T% of (>98%), superior dilutability, percent of drug content (>99%) and lowest leakage of the drug (in the 1st hour). Finally, the optimized produced NE formulations can be thought of as a unique way to improve the transport of PHCI molecules efficiently by overcoming the barrier qualities of the skin in (IH), and to be formulated as an emulgel dosage that shall be clinically investigated.

Keywords: Infantile hemangioma, Propranolol hydrochloride, Nanoemulsion.

¹² Corresponding author email: taif.abd2100n@kopcharm.uobaghdad.edu.iq
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Introduction

Infantile hemangiomas (IHs) are the most common vascular tumors of childhood which are benign but can cause significant functional and cosmetic morbidity (1), as shown in Figure 1. Hemangioma lesions have a predictable course of growth, but little is understood about the mechanism behind their development (2).

Infantile hemangioma believed to be a result of dysregulation of both vasculogenesis and angiogenesis (3); however, the triggers that initiate the development of infantile hemangioma are still a matter of debate. No single hypothesis is sufficient to describe all features of infantile hemangioma. The most likely scenario would involve hypoxic stress as the triggering signal, inducing overexpression of angiogenic factors such as vascular endothelial growth factor (VEGF) via the HIFα pathway (3).

Specific risk factors, such as low birth weight, prematurity, fertility medications, female gender, white race, and family history are associated with (IH) development. Untreated IH can result in significant physical disfigurement due to ulceration and scarring of skin lesions (4).

Propranolol hydrochloride is a nonselective β-adrenergic receptor blocker (heat stable, white, crystalline solid, Odorless, molecular weight 295.8 g/mol, melting point 163-165°C, pKa 9.5, Log P 1.2., readily soluble in water and ethanol), initially introduced to treat arrhythmias. In 2008, oral delivery of PHCl was found to be highly effective for the treatment of infantile hemangiomas (IH) (5), then it became the first-line medication and the only approved FDA medication for IH cure by the name of Hemangeol.

Figure 1. An illustration of the vessel grouping that causes infantile hemangioma and a child with an infantile hemangioma above his eye

The effect of PHCl on IH can be attributed to molecular mechanisms: vasoconstriction, decreased expression of VEGF and basic fibroblast growth factor (bFGF) genes through the down-regulation of the RAF-mitogen-activated protein kinase pathway, inhibition of angiogenesis, and induction of apoptosis, overall PHCl appears to be effective in treating infantile hemangiomas by reducing the size and growth of the tumor (6).

Systemic treatment with PHCl may cause severe complications and infants need to be closely monitored, as the systematic exposure to PHCl may induce changes in sleep patterns, acrocyanosis, and gastrointestinal symptoms, and even serious adverse reactions including bronchospasm, symptomatic hypotension, hypoglycemia, and bradycardia (3),(5),(7).

In a 40 infant patients systematic review, one case had developed serious tachycardia within the first 48 hours after propranolol was started orally, which had to terminate the treatment immediately (6).
Topical propranolol nanoemulsion

Figure 2. Natural development of infantile hemangioma

In contrast, dermal application of PHCl generates less untoward effects compared with the oral route for treating superficial hemangiomas, benefiting from high local drug concentration and less systemic exposure (8).

Nanoemulsion is clear, transparent/translucent, thermodynamically and kinetically stable system with a mean globule diameter of <100 nm (9). Nanoemulsions are considered as heterogeneous system in which dispersion of two non-miscible liquids in form of oil-in-water (o/w) or water-in-oil (w/o) that stabilized by an interfacial layer of surfactant (emulsifier) and co-surfactant (co-emulsifier) (10). Their appealing attributes, such as simple manufacturing, improved loading abilities, and long-lasting stability, position them as promising vectors for topical application (11).

Without any treatment, residual consequences occur in 70% of cases, namely telangiectasia as in Figure. 3-B, excessive fibrofatty tissue as in Figure. 3-C, and skin laxity due to the destruction of elastic tissue. Up to 12% of infantile hemangioma cases referred to pediatric centers are reported to be complex and prone to complications. The type and extent of complications depend on localization and size of the hemangioma, as well as the age of the infant. Previously ulcerated lesions usually leave scars (3).

Figure 3. (A) Hemangioma on right lower arm, age 14 weeks. (B) Residual telangiectasia at age 23 months. (C) Fibrofatty hemangioma residuum on the arm of a 4-year-old child.
Materials and Methods

Materials
Propranolol hydrochloride powder and Tween 20 were procured from Awamedica pharmaceuticals factory, Erbil, Iraq. Clove oil was purchased from_alpha Chemika, CO., India. PEG400 was provided from Sigma Aldrich, USA and PG from Panreac, CO., Barcelona, Spain. All other chemicals and solvents were of analytical reagent appraisal.

Methods

Powder X-ray diffraction (PXRD)
The crystalline state of pure PHCl was assessed using powder x-ray diffraction (DX2700BH, China). The parameters were set with target metals Cu, filter Kα, 40kV, and 30mA. The scan covered a 2θ range from 5-80° at a wavelength of 1.5406 Å. This diffraction pattern, unique to each crystal structure, was then recorded and analyzed (12).

Differential scanning calorimeter (DSC)
The thermal behavior and thermotropic properties of PHCl powder were evaluated using differential scanning calorimetry (DSC/TA-60 instrument from Shimadzu, Japan), equipped with the intercooler 2 cooling system. Nitrogen was utilized as a blank gas, and samples weighing 3-5 mg were heated in aluminum pans with scanning temperatures ranged from 50-250 °C at a scanning rate of 10 °C per minute (13).

Fourier Transform Infrared (FTIR)
To characterize PHCl using FTIR technique, FTIR spectrometer (IRAffinity-1, Shimadzu, Japan) was employed, which directly measured the absorption of infrared radiation by the sample in the range of 4000-400 cm⁻¹ without requiring sample preparation by KBr. The resulting spectrum was analyzed to identify the characteristic peaks of PHCl (14).

Saturation solubility study
The saturated solubility of PHCl was measured in distinct oils (sesame, castor, clove, anise, peppermint, oleic acid, triacetin and Capryol 90 oil), surfactants (Tween 20, Tween 80, and cremophor EL), co-surfactants (ethylene glycol, propylene glycol, PEG 400, ethanol, and transcutol P) and acetate buffer. An excess of PHCl powder was combined with exactly 5g of oils, surface active agents or co-surfactants in a 10g glass tube using a Vortex mixer then sonicated for 5min. The tube was securely sealed and agitated for 72 hours at a consistent temperature of 25 ± 1 °C using an isothermal shaker water bath (15).

Samples were centrifuged at 3000 rpm for 15 minutes, and then filtered using a 0.45-micrometer Millipore filter. They were diluted with methanol for UV analysis. Solubilization was measured spectrophotometrically at the drug’s λmax(290nm) in methanol, with methanol as a blank. Measurements were done in triplicate (16).

Pseudo-ternary phase diagram
The oil:S-mix (surfactant:co-surfactant) was combined at various weight ratios in different glass vials (ranging from 9:1, 8:2, 7:3, 6:4, . . . 2:8, 1:9) using a vortex for 5 minutes to create a homogeneous mixture. Then, deionized distilled water (DI DW) was titrated with each proportion at 25°C while continuously stirring and recording the amount of water added until the mixture become turbid as an endpoint (17).

Preparation of nanoemulsion
The low energy method of emulsification (aqueous titration method) was used to create the nanoemulsions. Ten unique blends were made using 0.375% (w/w) PHCl, resulting in a final nanoemulsion concentration of 3.75mg/1g. PHCl dissolved in oil phase and thoroughly mixed and sonicated by ultrasonication for 15-min to secure the drug in oil and prevent any possible later on migration of PHCl to aqueous phase, afterward the drug-oil combined with the S-mix, vortexed for 5 minutes, then slowly titrated with the aqueous phase until a clear emulsion formed (18).

Thermodynamic stability study
The formula's stability was evaluated by undergoing thermodynamic tests, including, heat-cold cycles, centrifugation and freeze-thaw evaluations (19).

Heating-cooling test
In the heating-cooling test, all ten formulations were heated at 45 °C and then cooled at 4 °C, with a duration of 24 h at each temperature, for 2 cycles to assess precipitation, cracking, and phase separation effects on stability. Successful ones underwent further thermodynamic tests.

Centrifugation
Each sample endured 15-min centrifugation 3500 rpm, for signs of cloudiness, phase shifts, or sedimentation. Formulations that cleared the first two stability tests were then freeze-thaw tested.

Freezing-thawing test
Finally, only formulations which passed the previous two steps were stored at alternating temperature of -21 and 25 °C, with duration of 24 h at each temperature, for 2 cycles. Physical changes were checked visually to gauge freeze-thaw stability.

Evaluation of nanoemulsion
pH measurement
To ensure that the developed topical nanoemulsion is compatible with the skin's pH and to avoid irritation upon application, a pH measurement test was conducted at room temperature (25±1°C) after 24 h without dilution.
For all the prepared o/w nanoemulsions using a digital pH meter (Hanna Instruments RI 02895 Romania), that had been calibrated with standard buffer solutions before use. The experiment was carried out in triplicate.

**Electrical conductivity**

To confirm the continuous phase-type and check for phase inversion phenomena, the conductivity measurement of each PHCl o/w nanoemulsion formulation was conducted, by immersing metal electrodes in a 20 g sample of the prepared formula at room temperature. The digital conductivity meter (TDS & EC meter, USA) was utilized [21].

**Globule size and Polydispersity index (PDI)**

To ascertain the size of the NE droplets, the fluctuations in light scattering due to Brownian motion of the particles was analyzed using the dynamic light scattering technique (Zeta-sizer Nano). Prior to measurement, the nanoemulsion was delicately stirred following a dilution with deionized water to enhance homogeneity [22]. The polydispersity index (PDI) was measured, in order to determine the homogeneity in globules size. The PDI is calculated by dividing the standard deviation by the mean droplet size and ranges from 0 to 1 [23].

**Percent of light transmittance (T%)**

The optical clarity of NE formulas was determined and spectrophotometric analysis was conducted spectrophotometer by utilizing a UV-Visible. The % transmittance at 650 nm was used as a measure of optical clarity for the developed NE formulas. Deionized water was used as a standard blank solution [24]. The equation for measuring light transmittance is:

\[ T\% = 10^{(-A)} \times 100 \]

Where T% is the light transmittance percentage and A is the absorbance of the sample.

**Dye solubility test**

To 5g of an o/w NE formula, 1-2 drops of 0.1% water-soluble dye methyl orange solution were added. The dye dispersed evenly throughout the globule. However, when added to a w/o globule, the same dye remains in the dispersed phase and does not spread evenly [25].

**Dilution test**

The Dilution Test is a widely used method to evaluate the stability of oil-in-water emulsions. This test is a critical quality assessment parameter for emulsion-based products in the pharmaceutical industries, and is regulated by pharmacopeias [26]. According to the United States Pharmacopeia (USP) the nanoemulsion should remain clear when diluted with 20 parts of water. If the nanoemulsion breaks before 20 parts of water are added, it is considered unstable.

**Drug content measurement**

Drug content measured by diluting (100 µL) of each sample with methanol at a ratio of 1:1000. The samples were sonicated for 15 minutes to ensure complete mixing; the samples were then filtered using a 0.45 µm filter syringe and acquired a clear solution. The drug content was measured using a UV/Vis spectrophotometer with PHCl λmax [27].

**Viscosity measurement**

Viscosity is a very leading parameter in the election of the optimum formula, as it gives inspection to residence time on the skin [28]. The digital viscometer (NDJ-5S, U.K., spindle no.1) was used to measure the viscosity of the nanoemulsion. The spindle of the viscometer was inserted in a graduated cylinder containing 10g of the prepared NE sample, and different rotation speeds of 6, 12, 30, and 60 rpm were used at a temperature of 25± 1 °C [29].

**In-vitro drug leakage test study**

The study was conducted by utilizing the Franz diffusion cell system. This system has a 12 mL cell volume receptor section with effective diffusion area equal to 1.767 cm², separated from the donor section by a synthetic semipermeable membrane, acting as a diffusional barrier (M.wt cut off 8,000-14,000 Dalton). Before use, the dialysis membrane was soaked in acetate buffer saline pH 5.6 (skin pH) for 24 hours. The prepared PHCl NEs were placed in the donor compartment of the cell with stirring fixed at 50 rpm at 37 ± 0.5 °C [30]. At fixed time intervals (5, 10, 15, 30, 45, 60, 90, 120, and 150 minutes), 1mL samples were extracted from the receptor compartment, filtered through a 0.45 µm filter syringe and replaced with an equivalent volume of fresh acetate buffer to maintain the sink condition. These samples then quantified using UV-visible spectroscopy at the drug’s λmax (288nm) in acetate buffer [31].

**Zeta potential ζ**

To assess the droplet charge (zeta potential) of the chosen nanoemulsion (NE) formula, the dynamic light scattering method (Zeta-sizer Nano ZS) was employed [32].

**Drug-excipient compatibility study**

By utilizing FTIR, the study revealed if there is any mark of interaction between dissolved PHCl in clove oil and the other components used in the construction of the optimum NE formula [33].

**Atomic force microscopy (AFM)**

AFM, a sophisticated tool, allows detailed surface examination under various conditions. It’s a great complement to SEM.
imaging, by accurately measuring NE globule size, provides 3D resolution in any environment and a histogram of the globule size distribution (34).

**Stability study**

The 10-gram samples of drug loaded NE of the chosen formula were enclosed in photoprotective glass tube and then placed in stability chambers at different temperature conditions i.e., room temperature condition (25 ± 5 °C) and accelerated condition (40 ± 5 °C) for 2 months. Duplicate samples are withdrawn at the 0, 1st and 2nd month to evaluate their appearance, pH, rheological behavior and drug content. The physical stability was assessed by visual inspection for physical changes such as phase separation and drug precipitation. Chemical stability is expressed as the content of drug determined by UV visible spectroscopic (31).

**Results and Discussion**

**Powder X-ray diffraction findings**

The findings in this study were found to be identical to those reported by a previous researchers regarding Rita Ambrus et al 2014 (12), that Propranolol Hydrochloride is a crystalline solid with a well-defined crystal structure. The characteristic peaks of PHCl appear at diffraction angles 2θ of 12.510° and 17.195° as displayed in Figure. 4.

![Figure 4. XRPD spectra of PHCl](image)

**Differential scanning calorimeter results**

Figure. 5 shows the DSC thermogram of pure (PHCl). It displays a sharp endothermic peak at 164.57°C, which indicates the melting point. This peak confirms that the drug is in pure crystalline form.

![Figure 5. PHCl thermogram](image)
**Fourier Transform Infrared rationalization**

By analyzing the FTIR spectrum of PHCl, it was able to identify the most characteristic functional groups present in the molecule. These specific functional groups which were identified in this study were found to be identical to those reported by "Clarke's Analysis of Drugs and Poisons" (35), such as the hydroxyl group representing at 3275.13 cm\(^{-1}\) and a sharp, distinct peaks at 1107.14 cm\(^{-1}\), 1577.77 cm\(^{-1}\), 771.53 cm\(^{-1}\), 1265.30 cm\(^{-1}\), 1242.16 cm\(^{-1}\) and 798.53 cm\(^{-1}\) as pointed out in Table 1 and Figure 6.

![FTIR spectra of PHCl](image)

**Figure 6. FTIR spectra of PHCl**

**Table 1. Characteristic peaks of pure propranolol hydrochloride**

<table>
<thead>
<tr>
<th>NO.</th>
<th>Functional groups and vibration</th>
<th>Indicative (35) value wave number (cm(^{-1}))</th>
<th>Analyzed value wave number(cm(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hydroxyl (O-H) stretching</td>
<td>3273 cm(^{-1})</td>
<td>3275.13 cm(^{-1})</td>
</tr>
<tr>
<td>2</td>
<td>Alkyl chain (C-H) stretching</td>
<td>2925 cm(^{-1})</td>
<td>2924.09 cm(^{-1})</td>
</tr>
<tr>
<td>3</td>
<td>Aromatic ring (C=C) stretching</td>
<td>1580 cm(^{-1})</td>
<td>1577.77 cm(^{-1})</td>
</tr>
<tr>
<td>4</td>
<td>Aromatic ring (C-H) bending</td>
<td>1405 cm(^{-1})</td>
<td>1400.32 cm(^{-1})</td>
</tr>
<tr>
<td>5</td>
<td>(C-O) stretching</td>
<td>1270 cm(^{-1})</td>
<td>1265.30 cm(^{-1})</td>
</tr>
<tr>
<td>6</td>
<td>(C-N) stretching</td>
<td>1240 cm(^{-1})</td>
<td>1242.16 cm(^{-1})</td>
</tr>
<tr>
<td>7</td>
<td>(C-O) bending</td>
<td>1103 cm(^{-1})</td>
<td>1107.14 cm(^{-1})</td>
</tr>
<tr>
<td>8</td>
<td>Alkyl chain (-CH3) bending</td>
<td>795 cm(^{-1})</td>
<td>798.53 cm(^{-1})</td>
</tr>
<tr>
<td>9</td>
<td>Chloride ion (-Cl) stretching</td>
<td>772 cm(^{-1})</td>
<td>771.53 cm(^{-1})</td>
</tr>
</tbody>
</table>

**Saturation solubility study outcomes**

Propranolol Hydrochloride exhibited the highest solubility in Clove oil (41.42 mg/g) as an oil phase among other oils that have been tested, among other surfactant, co-surfactant and dissolution median.

Table 2 shows the results of PHCl saturated solubility in various surfactants and co-surfactants. To prepare a stable nanoemulsion formulation, the solubility data obtained indicated the use of clove oil as the oil phase, Tween 20 as the surfactant, and PEG400 and propylene glycol as co-surfactants.

**Generations of pseudo-ternary phase diagrams**

Built on the solubility study, clove oil was selected as an oil phase, tween 20 was chosen as surfactant and (PEG400 and PG) as a co-surfactant. S-mix ratios was 1:1, 2:1 and 3:1 for S-mixtures, (tween20/PEG400) in the red area, 2:1 and 3:1 for (tween20/PG) in the yellow area, as displayed in Figure 7.

**Preparation and selection of PHCl nanoemulsion**

The nanoemulsion region in the pseudo-ternary phase diagram has many possible formulations to be made. The oil phase percentage can be up to 12.5% w/w while maintaining a desirable globule size of less than 100 nm. The best oil phase for the formulation should completely solubilize the drug dose. This is another criterion that is taken into consideration when choosing the oil phase. To avoid any irritation and safely deliver the drug, a low concentration of a non-ionic surfactant, Tween 20, was preferred in the formulations. Water percentage was mininized in each formula equal to PHCl percentage (0.375%). Table 3 represent the composition of each prepared formula.

Table 3 represent the composition of each prepared formula.
### Table 2. Saturated solubility study results

<table>
<thead>
<tr>
<th>Sample</th>
<th>solubility (mg/g) mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oils</strong></td>
<td></td>
</tr>
<tr>
<td>Sesame oil</td>
<td>5.51 ± 0.83</td>
</tr>
<tr>
<td>Castor oil</td>
<td>1.31 ± 0.13</td>
</tr>
<tr>
<td>Clove oil</td>
<td>41.42 ± 2.12</td>
</tr>
<tr>
<td>Peppermint</td>
<td>9.43 ± 0.89</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>11.47 ± 0.69</td>
</tr>
<tr>
<td>Triacetin oil</td>
<td>0.93 ± 0.11</td>
</tr>
<tr>
<td>Capryol 90</td>
<td>7.97± 0.92</td>
</tr>
<tr>
<td><strong>Surfactants</strong></td>
<td></td>
</tr>
<tr>
<td>Tween 20</td>
<td>13.12 ± 1.05</td>
</tr>
<tr>
<td>Tween 80</td>
<td>11.77 ± 0.81</td>
</tr>
<tr>
<td>Cremaphor EL</td>
<td>9.82 ± 0.98</td>
</tr>
<tr>
<td><strong>Co-surfactants</strong></td>
<td></td>
</tr>
<tr>
<td>Ethylene glycol</td>
<td>117.47 ± 3.48</td>
</tr>
<tr>
<td>Propylene glycol PG</td>
<td>54.94 ± 2.31</td>
</tr>
<tr>
<td>PEG400</td>
<td>46.06 ± 1.93</td>
</tr>
<tr>
<td>Ethanol</td>
<td>41.65 ± 3.17</td>
</tr>
<tr>
<td>Transcutol P</td>
<td>27.71 ± 1.15</td>
</tr>
<tr>
<td><strong>Dissolution medium</strong></td>
<td></td>
</tr>
<tr>
<td>Acetate buffer pH5.6</td>
<td>136.42 ± 2.76</td>
</tr>
</tbody>
</table>

![Nanoemulsion phase diagrams for clove oil, DI DW and either (S-mix Tween20:PEG400 (red area)) or (S-mix Tween20:PG (yellow area))](image)

Figure 7. Nanoemulsion phase diagrams for clove oil, DI DW and either (S-mix Tween20:PEG400 (red area)) or (S-mix Tween20:PG (yellow area))
**Table 3. Composition (w/w %) of PHCl (0.375%) NE formulations**

<table>
<thead>
<tr>
<th>Formula code</th>
<th>Formula No.</th>
<th>S-mix ratio</th>
<th>C1 S-mix % w/w</th>
<th>Clove Oil % w/w</th>
<th>PHCl % w/w</th>
<th>DI DW % w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>F-1</td>
<td>1:1</td>
<td>60</td>
<td>10</td>
<td>0.375</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>F-2</td>
<td>1:1</td>
<td>62.5</td>
<td>12.5</td>
<td>0.375</td>
<td>25</td>
</tr>
<tr>
<td>PEG400 Based</td>
<td>F-3</td>
<td>2:1</td>
<td>60</td>
<td>10</td>
<td>0.375</td>
<td>30</td>
</tr>
<tr>
<td>Based</td>
<td>F-4</td>
<td>2:1</td>
<td>62.5</td>
<td>12.5</td>
<td>0.375</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>F-5</td>
<td>3:1</td>
<td>60</td>
<td>10</td>
<td>0.375</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>F-6</td>
<td>3:1</td>
<td>62.5</td>
<td>12.5</td>
<td>0.375</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>F-7</td>
<td>2:1</td>
<td>60</td>
<td>10</td>
<td>0.375</td>
<td>30</td>
</tr>
<tr>
<td>T2</td>
<td>F-8</td>
<td>2:1</td>
<td>62.5</td>
<td>12.5</td>
<td>0.375</td>
<td>25</td>
</tr>
<tr>
<td>PG Based</td>
<td>F-9</td>
<td>3:1</td>
<td>60</td>
<td>10</td>
<td>0.375</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>F-10</td>
<td>3:1</td>
<td>62.5</td>
<td>12.5</td>
<td>0.375</td>
<td>25</td>
</tr>
</tbody>
</table>

**Thermodynamic stability results**

The research used stress tests to create a stable nanoemulsion that avoided phase separation, drug precipitation, and cloudiness. All the formulations passed centrifugation, heating-cooling, and freeze-thaw tests, proving their thermodynamic stability. The results suggest the nanoemulsions were physically and thermodynamically stable. No flocculation, aggregation, phase separation, creaming, or coalescence occurred when the surfactant, cosurfactant, oil, and water were mixed in the specific quantities used in the study, which presented in Table 4.

**Table 4. Results of thermodynamic stability study of PHCl NE**

<table>
<thead>
<tr>
<th>Formula code</th>
<th>Formula No.</th>
<th>Heating–cooling cycles</th>
<th>Centrifugation test</th>
<th>Freeze–thawing cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1 PEG400 Based</td>
<td>F-1</td>
<td>Passed</td>
<td>Passed</td>
<td>Passed</td>
</tr>
<tr>
<td></td>
<td>F-2</td>
<td>Failed</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td></td>
<td>F-3</td>
<td>Passed</td>
<td>Passed</td>
<td>Passed</td>
</tr>
<tr>
<td></td>
<td>F-4</td>
<td>Passed</td>
<td>Passed</td>
<td>Passed</td>
</tr>
<tr>
<td></td>
<td>F-5</td>
<td>Passed</td>
<td>Passed</td>
<td>Passed</td>
</tr>
<tr>
<td></td>
<td>F-6</td>
<td>Passed</td>
<td>Passed</td>
<td>Passed</td>
</tr>
<tr>
<td>T2 PG Based</td>
<td>F-7</td>
<td>Passed</td>
<td>Passed</td>
<td>Passed</td>
</tr>
<tr>
<td></td>
<td>F-8</td>
<td>Failed</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td></td>
<td>F-9</td>
<td>Passed</td>
<td>Passed</td>
<td>Passed</td>
</tr>
<tr>
<td></td>
<td>F-10</td>
<td>Passed</td>
<td>Passed</td>
<td>Passed</td>
</tr>
</tbody>
</table>

**Nanoemulsion analysis evaluation**

**pH measurement results**

The pH of the developed formulations is shown in Table 5, which ranges from 6.39 to 7.15 with no significant difference among them (P>0.05), indicating the compatibility and safety of all the formulations for topical application, so reduce the irritation upon instillation.

**Conductivity test results**

A rise in oil content in the NE formula led to lower electrical conductivity at the same S-mix ratio. For instance, with F5 and F6, conductivity fell from 79 to 50 µS/cm. Conversely, a higher surfactant ratio increased conductivity, as seen in F7 and F9. The test results in Table 5, ranging from 50–91 µS/cm, verified the successful preparation of o/w nanoemulsions.

**Globule size and polydispersity index (PDI)**

The size of nanoparticles is crucial for enhancing the depth and rate of dermal penetration of drugs. As shown in Table 5, the average globule size of the prepared formulations ranged from (14.43 to 91.06) nm, indicating that all globules were nanosized. Increasing the oil content resulted in an increase in globule size, consistent with previous studies that showed the expansion of oil droplets in the nanoemulsion would increase the mean globule dimension like in F3 (59.72nm) and F4 (89.05nm). This is due to a decrease in the concentration of Smix in the formulations as more oil is added. The majority of the prepared nanoemulsion systems were in the nanosized range, possibly due to the penetration of cosurfactant molecules into the surfactant film. This penetration reduces the interfacial film surface viscosity, which then decreases the radius of curvature of the particles. As a result, transparent systems are formed.
Table 5. pH, electrical conductivity, globule size, PDI and dilution test findings

<table>
<thead>
<tr>
<th>Formula NO.</th>
<th>pH</th>
<th>Conductivity µS/cm</th>
<th>Globule size nm</th>
<th>PDI</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Code: T1-PEG400</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F1</td>
<td>6.48 ± 0.04</td>
<td>69</td>
<td>---</td>
<td>---</td>
<td>Failed</td>
</tr>
<tr>
<td>F2</td>
<td>6.39 ± 0.02</td>
<td>53</td>
<td>---</td>
<td>---</td>
<td>Failed</td>
</tr>
<tr>
<td>F3</td>
<td>6.54 ± 0.03</td>
<td>74</td>
<td>47.78 ± 0.61</td>
<td>0.385 ± 0.06</td>
<td>Passed</td>
</tr>
<tr>
<td>F4</td>
<td>6.49 ± 0.01</td>
<td>52</td>
<td>---</td>
<td>---</td>
<td>Failed</td>
</tr>
<tr>
<td>F5</td>
<td>6.63 ± 0.02</td>
<td>79</td>
<td>14.57 ± 0.25</td>
<td>0.289 ± 0.008</td>
<td>Passed</td>
</tr>
<tr>
<td>F6</td>
<td>6.51 ± 0.02</td>
<td>50</td>
<td>---</td>
<td>---</td>
<td>Failed</td>
</tr>
<tr>
<td>Code: T2-PG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F7</td>
<td>7.11 ± 0.01</td>
<td>78</td>
<td>58.42 ± 0.51</td>
<td>0.549 ± 0.05</td>
<td>Passed</td>
</tr>
<tr>
<td>F8</td>
<td>7.09 ± 0.03</td>
<td>66</td>
<td>---</td>
<td>---</td>
<td>Failed</td>
</tr>
<tr>
<td>F9</td>
<td>7.15 ± 0.03</td>
<td>91</td>
<td>23.39 ± 2.63</td>
<td>0.380 ± 0.02</td>
<td>Passed</td>
</tr>
<tr>
<td>F10</td>
<td>7.13 ± 0.02</td>
<td>58</td>
<td>---</td>
<td>---</td>
<td>Failed</td>
</tr>
</tbody>
</table>

Percentage of transmittance

Table 6 shows the percentage of light transmittance of the nanoemulsion formulations. All of the formulations have a high percentage of light transmittance, indicating that they are clear and transparent due to small globular size.

Table 6. Drug content and light transmittance results

<table>
<thead>
<tr>
<th>Formula NO.</th>
<th>Drug content %</th>
<th>Light transmittance T%</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>99.21 ± 0.45</td>
<td>98.78 ± 0.23</td>
</tr>
<tr>
<td>F2</td>
<td>99.74 ± 0.72</td>
<td>97.91 ± 0.37</td>
</tr>
<tr>
<td>F3</td>
<td>98.63 ± 0.39</td>
<td>98.43 ± 0.14</td>
</tr>
<tr>
<td>F4</td>
<td>100.02 ± 0.71</td>
<td>97.80 ± 0.48</td>
</tr>
<tr>
<td>F5</td>
<td>99.79 ± 0.44</td>
<td>99.09 ± 0.13</td>
</tr>
<tr>
<td>F6</td>
<td>98.68 ± 0.36</td>
<td>98.19 ± 0.18</td>
</tr>
<tr>
<td>F7</td>
<td>98.73 ± 0.23</td>
<td>98.71 ± 0.31</td>
</tr>
<tr>
<td>F8</td>
<td>98.75 ± 0.31</td>
<td>99.96 ± 0.25</td>
</tr>
<tr>
<td>F9</td>
<td>99.13 ± 0.55</td>
<td>99.17 ± 0.16</td>
</tr>
<tr>
<td>F10</td>
<td>98.76 ± 0.34</td>
<td>97.18 ± 0.29</td>
</tr>
</tbody>
</table>

Drug content results

The drug content findings were satisfactory, signifying that PHCl was loaded successfully in oil phase of all the prepared formulations with homogeneity and stability, and without drug precipitation. Table 6 displays the results, which demonstrated that all of the formulated NEs met the British Pharmacopoeia range requirement of 95% to 110%.

Solubility of Dye in nanoemulsion

It was found that the dye was uniformly distributed throughout the continuous phase of the PHCl o/w NE, as presented in Figure. 8.

Dilution capacity findings

In this test, all formulas were tested for dilution capacity and the results are shown in Table 5. Dilution Test Results showed that the oil content within the formula is connected to its ability to carry water at the same S-mix ratio. As the oil content increases, less water dilution capacity is seen. This is displayed in formula F7+F8 with S-mix 3:1 in Figure. 8.

Figure 8. Photographic pictures displaying dye solubility test formula F5 (left) and dilution test, F7 and F8 (right)
**Viscosity study analysis**

The study found that there was a positive correlation between the concentration of surfactant and the viscosity of the nanoemulsion. This is likely due to the surfactant molecules (Tween 20) forming a cross-linked structure that traps water molecules, leading to an increase in viscosity. (F5 is more viscous than F3), while an elevation in oil% will lead to viscosity thinning attitude (F9 is more viscous than F10 at S-mix 3:1). The highest viscosity observed at 6 rpm was (314.3 ± 1.36 mpa.s) and the lowest one was (216.5 ± 2.21 mpa.s) for (F5) and (F10) respectively. Additionally, it was observed that increasing the spindle speed or shear rate revealing the pseudoplastic flow behavior of the prepared NEs (shear thinning liquids) due to viscosity lessen (38). More, its shows that the formulas with PEG400 S-mix as a co-surfactant possess higher viscosity regarding those with PG as a co-surfactant. Figure. 9 represent of the discussed viscosity out comes in depth.

![Figure 9. Viscosities data of prepared PHCl NEs formulas (F3, F5, F6, F9 and F10)](image)

**In-vitro drug leakage test analysis**

The Observations of in-Vitro leakage reveal a desired duration of time for complete cumulative % of HPCL leakage from each formula, in which (F5) and (F9) formulation almost completely liberated (near100%) after 120 min, while (F3), needed 150 min for leakage of more than 95% of HPCL compared to the fast-pass of PHCl solution freshly prepared. The outcomes show that formulations with a higher S-mix ratio led to more leakage of PHCl. This could be attributed to the solubilizing effect and the increased hydrophilicity of PHCl, brought about by the use of Tween 20 surfactant in higher concentrations as the S-mix ratio elevated (39). Additionally, the formula with PEG400 (F5) as co-surfactant shows a better performance in comparison with that of PG (F9) as co-surfactant despite the same S-mix ratio (3:1), as shown in Figure.10.

The similarity factor, or f2, came out to be 22.807. This indicates that the performance profiles of the PHCl nanoemulsion (F5) and pure PHCl are not similar, while the leakage kinetics are believed to be comparable between the formulas (F3), (F5) and (F9) as f2 is more than 55 (40). Based on the highest R² values, the Korsmeyer-Peppas model is the best fit for describing the mechanism of PHCl leakage from NE formulations. This model has the highest R² values for all tested formulations, as shown in the Table 7.

![Figure 10. Leakage behaviors of PHCl NEs formulas (F3, F5, F9 and PHCl aqueous solution)](image)

**Table 7. Kinetic analysis data profile of PHCl from prepared NEs (F3, F5 and F9)**

<table>
<thead>
<tr>
<th>Formula</th>
<th>Zero order k₀</th>
<th>R²</th>
<th>First order K₁</th>
<th>R²</th>
<th>Higuchi model Kₕ</th>
<th>R²</th>
<th>Korsmeyer-Peppas model Kₖₚ</th>
<th>n</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>F3</td>
<td>0.744</td>
<td>0.9348</td>
<td>0.015</td>
<td>0.9640</td>
<td>7.581</td>
<td>0.9571</td>
<td>2.846</td>
<td>0.719</td>
<td>0.9854</td>
</tr>
<tr>
<td>F5</td>
<td>0.853</td>
<td>0.6512</td>
<td>0.025</td>
<td>0.9526</td>
<td>8.964</td>
<td>0.9740</td>
<td>10.845</td>
<td>0.462</td>
<td>0.9967</td>
</tr>
<tr>
<td>F9</td>
<td>0.824</td>
<td>0.7884</td>
<td>0.021</td>
<td>0.9699</td>
<td>8.578</td>
<td>0.9845</td>
<td>7.053</td>
<td>0.547</td>
<td>0.9897</td>
</tr>
</tbody>
</table>
As the result show a similar performance associated with lowest leakage with PHCI-loaded NEs (50% within 1st hour) in comparison with the aqueous solution that pass the same membrane quickly (100% within 30 minutes), both formula (F3 and F5) could be a good candidate for further characterizations. **Zeta potential ζ**

A zeta potential value as low as 30 mV can provide sufficient stabilization (41). The formula (F5), has chosen to be analyzed with zeta potential value of (-19.89 mV) as displayed in Figure. 11 along with its globular size, which enhance the stability of the nanoemulsion by preventing the individual globules from coalescing into larger globule due to repulsion.

**Figure 11. Average globule size and zeta potential of (F5) formula**

**Drug and excipient compatibility**

The PHCl molecules were mixed, solubilized, and dispersed solely in the clove oil (oil phase) where despite the inert nature for others excipients, there will be minor contact between S-mix and PHCl. FTIR analysis for PHCI-Clove oil had been done and the majority of PHCI peaks remain despite the noise and
fingerprint overlapping produced by both the concentration and the molecular size of clove oil. The study shows in Figure. 12 that the functional group band was not affected, albeit some of the stretching frequencies vanished due to PHCl's solubility in the oil and there was no chemical interaction between the drug functional groups and the main NE component.

![FTIR spectrum of the dissolved PHCl in clove oil](image)

**Figure 12.** FTIR spectrum of the dissolved PHCl in clove oil

**AFM size and morphology rationalization**

The size and morphology of the PHCl NE (selected formula) (F5) were confirmed by employing imaging with the high precision of the AFM. The findings approved that the NE globules are spherical in shape and fall within the nano range (42), as confirmed by the globule size distribution histogram and surface 3D view which presented in Figures. 13 and 14.

![Globule size analysis histogram by AFM of PHCl nanoemulsion (F5)](image)

**Figure 13.** Globule size analysis histogram by AFM of PHCl nanoemulsion (F5)

![Surface 3D view with images of PHCl nanoemulsion (F5) by AFM](image)

**Figure 14.** Surface 3D view with images of PHCl nanoemulsion (F5) by AFM

**Stability study findings**

The optimum nanoemulsion formulations stood the test of time, remaining unaltered and steadfast for a solid two months. Their physical charisma, pH balance, rheological characteristics, and drug content
remained untouched with no significant changes (P>0.05), declaring a high stamina.

**Conclusion**

Propranolol hydrochloride was successfully encapsulated in NEs system generated by spontaneous emulsification approach using clove oil, Tween 20, and PEG 400 by employing nano-technology.

The findings showed that more than one PHCl loaded nanoemulsions formula, such as (F3) and (F5), could be optimized for subsequent dosage form development because both of them had the following characteristics: globule size of (<50 nm), low PDI (0.3), light transmittance T% of (>98%), superior dilatability, percent of drug content (99%), and lowest leakage of the drug (in the first hour).

In conclusion, the outcomes of this research possess the desired topical characteristics and have the ability to be manufactured in the form of a nanoemulgel dosage form, with a high potential for application in the treatment of infantile hemangioma IH in clinical trials.

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**Conflicts of Interest**

The authors declare no conflicts of interest.

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**Ethics Statements**

The authors claim that, in accordance with the research integrity guidelines in Iraq, ethical approval from an ethics commission is not required.

**Reference**

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