Acta Pharm. Sci. Vol 62:(2), 2024
Research article
DOI: 10.23893/1307-2080.APS6229

Sprayable microemulsion of diphenhydramine hydrochloride for dermal delivery

Muhammet Davut ARPA[1*], Tuğba ARSLAN[1], Huriye ERASLAN[1], Neslihan ÜSTÜNDAĞ OKUR[2]

1 Istanbul Medipol University, School of Pharmacy, Department of Pharmaceutical Technology, Istanbul, Türkiye
2 University of Health Sciences, Faculty of Pharmacy, Department of Pharmaceutical Technology, Istanbul, Türkiye

ABSTRACT

In this work, a sprayable microemulsion of diphenhydramine hydrochloride (DPH HCl) was developed for use in conditions that cause itching, such as insect bites, mild sunburns, and skin irritations. Microemulsions were prepared using oleic acid and isopropyl myristate as the oil phase (1:2), Tween 20 and Transcutol HP as surfactants, isopropyl alcohol as co-surfactant, and water. The microemulsions (M1, M2, and M3) that presented greater area were selected as lead microemulsions and loaded with 2% DPH HCl (w/w). The physical stabilities of drug-loaded formulations with a droplet size of 15.998-19.030 nm, polydispersity index of 0.404-0.516, and turbidity of 2.41-2.50 Ntu were evaluated as appropriate. The microemulsions showed a great spread area in sprayability studies. Moreover, DPH HCl release from microemulsions reached 80-100% within one hour. The sprayable microemulsions were highly suitable for topical application of DPH HCl and can be evaluated for clinical applications with further studies.

Keywords: diphenhydramine hydrochloride, microemulsion, dermal delivery, topical application, topical spray

*Corresponding author: Muhammet Davut ARPA
E-mail: mdarpa@medipol.edu.tr
ORCIDs:
Muhammet Davut ARPA: 0000-0001-9290-2404
Tuğba ARSLAN: 0000-0002-7131-0570
Huriye ERASLAN: 0009-0002-7466-9842
Neslihan ÜSTÜNDAĞ OKUR: 0000-0002-3210-3747
(Received 5 Jan 2024, Accepted 6 Feb 2024)

INTRODUCTION

Microemulsions, characterized by small droplet sizes up to 200 nm, emerge as interesting and promising submicron carriers for dermal drug delivery owing to their thermodynamic stability, transparency, optically isotropic, and spontaneous formation[1,2]. Microemulsions are usually formed by the dropwise addition of a water phase to a mixture of oil, surfactant, and cosurfactant[3]. They exhibit low interfacial tension attributed to the presence of surfactants and these properties contribute to the maintenance of stability[4]. Microemulsions offer significant advantages including providing higher drug loading capacity by enhancing the solubility of the active ingredient, increasing the thermodynamic activity of the drug, and improving the permeability of the drug thanks to its components[5]. Additionally, it enables the combination of water-soluble and oil-soluble substances[6]. These formulations with low viscosity can be used in this form, as well as they can be designed as microemulsion-based gel with the addition of a gel-forming agent[7]. The solution-like low viscosity of microemulsions allows them to be designed as sprayable formulations[8] that provide high patient compliance in terms of application.

Diphenhydramine (DPH) was initially synthesized in 1943 by Dr. George Rieveschl from the University of Cincinnati and it was FDA's first approval for an antihistamine drug. DPH attempts as an antagonist of the H-1 receptor, counteracting the impact of histamine and relieving allergic reaction symptoms[9]. Its oral tablet and liquid preparations are commercially available on the market[10]. However, the short half-life (2-9 h) of DPH along with the first-pass effect resulting in low oral bioavailability (40-60%), necessitates frequent administration (from 25 mg to 50 mg, 3 to 4 times a day) to maintain the aimed plasma levels[11]. Since the high systemic side effects resulting from oral delivery will decrease patient compliance, dermal delivery, which has fewer systemic side effects, can be preferable[12]. DPH, this first-generation antihistamine, has perfect anesthetic and antipruritic effects when used topically[13]. Therefore, they effectively reduce itching caused by local allergic reactions to insect bites, mild sunburns, mild skin irritations, etc., and ensure good patient compliance with relief of pain and suffering[9,12].

Studies have been conducted on the topical application of diphenhydramine hydrochloride (DPH HCl), including microemulsion[12,14-17]. However, no sprayable microemulsion formulations of DPH HCl were found. This study aimed to develop a novel sprayable microemulsion formulation loaded with DPH HCl, which is the soluble salt of DPH, and propose an optimized formulation for treating seasonal allergies, insect bites, stings, and rashes. The physicochemical characterization and *in vitro* drug release profiling of the formulated microemulsions were investigated for this aim.

METHODOLOGY

Materials

DPH HCl, oleic acid, acetonitrile, and phosphate buffer tablet (PBS, pH 7.4) were purchased from Sigma-Aldrich, USA. Transcutol HP (diethylene glycol monoethyl ether, HLB 4.2) and Isopropyl myristate (IPM) were purchased from Alfa Aesar, USA. Tween 20 (polyoxyethylene sorbitan laurate, HLB 16.7) was purchased from Merck, Germany. Isopropyl alcohol (IPA) was purchased from Carlo Erba, Italy.

Solubility of DPH HCl

The solubility of DPH HCl was investigated at ambient temperature in various solvents, including distilled water, IPM, oleic acid, IPA, Transcutol HP, and Tween 20. The studies were performed with minor revisions to the previous study[18]. An excess amount of DPH HCl was dispersed in a microcentrifuge tube with one mL of the solvent. Each tube was shaken for 24 h in a horizontal shaker (SSL2, Stuart, UK), and then the mixture was centrifuged (3-18KS, Sigma, Germany) at 14,000 rpm for 30 min. The supernatant was subjected to dilution with the mobile phase (acetonitrile: water, 35:65, v/v) at different ratios and subsequently analyzed by an HPLC method. The same procedure was separately carried out for each solvent.

An HPLC method was developed and validated for the quantification of DPH HCl. Analysis conditions were recorded on the HPLC device (1100 series, Agilent, USA) having a UV detector as in Table 1. A C18 column (InertSustain C18, 150x4.6mm, 5µm, GL Sciences, Japan) was used during the analysis.

Table 1. Conditions of the HPLC method for the quantification of DPH HCl

HPLC condition	
Mobile phase	Acetonitrile: pH 3 phosphate buffer (35:65, v/v)
Flow rate	1.2 mL/min
Wavelength	225 nm
Column temperature	30 °C
Injection volume	20 µL

Preparation of microemulsion formulations

Pseudo-ternary phase diagrams were meticulously created to determine an optimal concentration of the components within a microemulsion design. In this diagram, typically one corner represented a mixture of surfactants, while the other corners were the oil and water components. All the formulation components were mixed in proportions ranging from 0 to 100%. Then, each system was visually observed, and the resulting phase boundaries were drawn[19]. During the studies, various substances were used in different concentrations. As an oil mixture, jojoba oil, IPM, and oleic acid were studied in different ratios and pairs. It was observed that formulations containing jojoba oil did not result in a stable microemulsion. Consequently, in the preparation of microemulsion systems, IPM and oleic acid were selected as the oil phase, Transcutol HP and Tween 20 as the surfactants, and IPA as a co-surfactant.

For preliminary studies, the surfactant and co-surfactant were mixed in different ratios (w/w), namely 3:1, 2:1, 1:1, 1:2, and 1:3. The oil phase and the mixture of surfactant were stirred within a beaker on a magnetic stirrer (Mr-Hei Standard, Heidolph, Germany) at 300 rpm until the mixture homogenized. These mixtures were titrated, drop-by-drop, with first IPA and then distilled water. Addition of distilled water was maintained until the mixture became blurred. While all processes were carried out at ambient temperature, the appearance of the formulation was followed visually. After marking the values that ensure the formation of a clear microemulsion in the phase diagram, the center of the formed microemulsion region was accepted as the lead microemulsion. Drug-loaded formulations were prepared as before. DPH HCl (2%, w/w) was added to the lead microemulsion at the last stage and dissolved by stirring at 300 rpm for 15 min.

Characterization of microemulsion formulations

Characterization studies carried out to assess the suitability of the drug-loaded and blank microemulsions were carried out at least three times.

Viscosity

The viscosities of formulations were measured by a vibrational viscometer (SV-10, AND Vibro Viscometer, Japan)[20]. A sample of 10 mL microemulsion was filled into a plastic container, and measurements were taken at room temperature until the value was constant. The viscosity studies of each batch were repeated three times, and each measurement was conducted twice.

Droplet size, polydispersity index (PDI), and conductivity

Average droplet size, PDI, and conductivity of the drug-loaded and blank microemulsions were determined using the dynamic light scattering method by a zetasizer (Zetasizer Ultra, Malvern Instruments, Worcestershire, UK) at ambient temperature[21,22]. Disposable cuvettes were used for the measurement of the droplet size and PDI of the formulations. Folded capillary cells were used for the determination of the conductivity of the microemulsions.

Turbidity, refractive index, and physical stability

The turbidity of the formulations was assessed using a digital turbidity meter (TB 210 IR, Lovibond, UK) The instrument was calibrated using solutions of varying turbidity levels specific to the instrument. Subsequently, the microemulsion was filled into a glass bottle up to the marked line and the measurement was performed upon insertion into the device. The refractive index was determined using a digital refractometer (DR 301-95, Kruss, Germany)[23]. Physical stability tests were performed under temperature and centrifuge conditions to determine whether there were changes in the physical stability of the microemulsions such as phase separation or sedimentation. Six cycles of cooling (5±1°C)-heating (45±2°C) conditions were applied to the microemulsions. Additionally, the microemulsions were centrifuged (3-18KS, Sigma, Germany) at 25 °C and 5,000 rpm for 30 min. At the end of the procedures, the microemulsions were assessed visually[7].

Sprayability

A sample of 10 g from each formulation was separately weighed into a spray bottle. Subsequently, a certain amount of a dye (E133) was added to the bottles, and the formulation was stirred using a magnetic stirrer at 500 rpm for 45 min. Following our previous studies[24], a texture analyzer (TA.XT.*Plus*C, Stable Micro Systems, Haslemere, Surrey, UK) was employed for sprayability studies. As shown in Figure 1, a white plate was horizontally positioned 15 cm away from the bottle, and the spray process was conducted five times for each sample. After the process, the diameter of the spray area on the plate was measured.

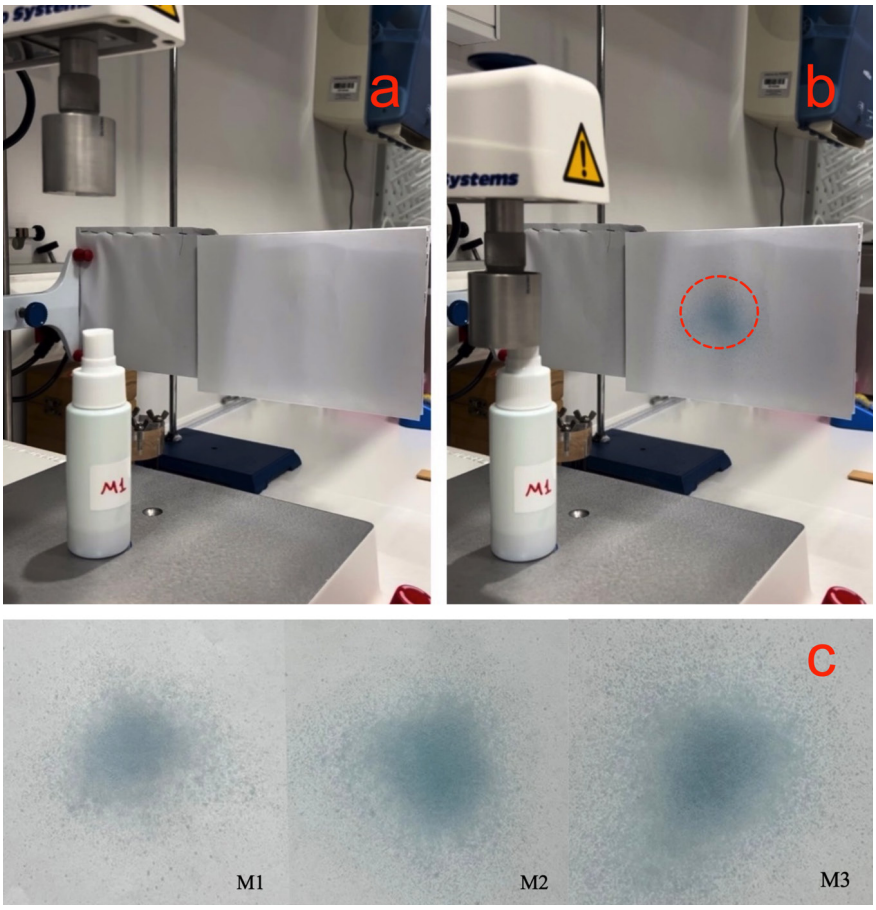

Figure 1. Images of sprayability studies. (a) Before the spray process. (b) After the spray process. (c) The spreading area of M1, M2, and M3 formulations after the spray application

In vitro drug release

In order to evaluate the release profile of the formulations, the DPH HCl-loaded microemulsions were studied through the dialysis bag method, following the procedures from previous studies[22,24]. A sample of 0.5 g formulation was transferred to a dialysis bag. After the bag was tightly closed, it was placed in a beaker containing 200 mL of pH 7.4 phosphate buffer (PBS). The studies were conducted on a magnetic stirrer at 50 rpm and 32±2°C. At seven distinct time points (5, 10, 15, 20, 30, 45, and 60 min), one mL was withdrawn from the release medium using an injector. Following each sampling, one mL of fresh PBS was added to maintain a constant volume. The samples were analyzed by HPLC at 225 nm. The experiments were performed four times for each batch.

On the one hand, the release of DPH HCl from the formulations was evaluated utilizing model-independent approaches, which were the difference factor (f1) and similarity factors (f2)[25,26]. On the other hand, the release profiles were assessed using model-dependent kinetics such as zero order, first order, Higuchi, and Hixson-Crowell[22].

Statistical analyses

The data were presented as mean±SD. Statistical analysis was performed using a student's t-test.

RESULTS and DISCUSSION

Solubility of DPH HCl

The solubility of DPH HCl in various solvents was analyzed using HPLC. It was observed that all formulation components, except IPM, dissolved the active substance (Table 2). DPH HCl was found to be freely soluble in distilled water and Transcutol HP, while the solubility of DPH HCl was lowest in IPM (0.011 mg/mL) compared to other solvents.

Table 2. Solubility of DPH HCl in different solvents

Solvent	Concentration (mg/mL±SD)
Distilled water	622.358±2.515
IPA	23.541±1.018
Tween 20	59.131±2.111
Transcutol HP	129.706±3.001
Oleic acid	10.850±0.300
IPM	0.011±0.001

Preparation of microemulsion formulations

Microemulsions consist of four main components: water, oil, surfactant, and co-surfactant phases. In this project, IPM, chosen as the oil phase for the formulation of microemulsion systems, demonstrates a robust effect in enhancing permeation and biocompatibility[7,27]. Oleic acid, a monounsaturated fatty acid, is frequently used as the oily phase in the formation of microemulsions and is well-compatible with IPM[28,29]. The ratio of oleic acid to IPM was 1:2 (w/w) for all formulations. Transcutol HP, monoethyl ether of diethylene glycol, has been comprehensively studied as a penetration enhancer in various

transdermal therapeutic systems, with the potential to enhance drug solubility and improve drug delivery[30]. Additionally, Hernandez et al. found that a high concentration of Transcutol HP reduces the surface tension and leads to the formation of small droplets[31]. Polysorbate 20 or Tween 20, the most hydrophilic surfactant among polysorbates, is nonionic and biocompatible. This surfactant, a sorbitan monolaurate derivative ethoxylated with approximately 20 ethylene oxide units, has the effect of increasing the solubility capacity. Thanks to these properties, it is frequently used in microemulsion formulations[32,33].

The most crucial stage for the preparation of the microemulsions to acquire the desirable composition of the used compounds is to construct a pseudo-ternary phase diagram. These diagrams serve to determine the range of microemulsion existence and explore the influence of varying component weight ratios on the size of a stable microemulsion area[26]. The corners of the phase diagram represent the components of the microemulsion (water, oil, and surfactant: cosurfactant). Any point in the diagram reveals the proportions of these components that make up the formulation so that there is a total of 100%. The center of the area, where the microemulsion is formed, is considered to be the ideal microemulsion. The pseudo-ternary phase diagrams of the formulations were created using the Microsoft Excel program. Then, three formulations that provide the greatest microemulsion area were selected. Figure 2 shows the pseudo-ternary phase diagrams of the selected microemulsion formulations. The surfactant: cosurfactant ratios of M1, M2, and M3 formulations were 1:1, 1:2, and 1:3, respectively. Accordingly, the ideal formulations were determined and drug-loaded microemulsions were prepared by adding DPH HCl (2%, w/w) following the composition ratios (Table 3).

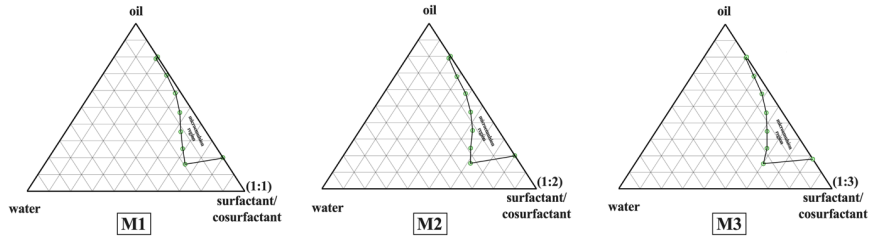

Figure 2. Pseudo-ternary phase diagram of selected microemulsions, which provide greater microemulsion region

Table 3. Composition of the drug-loaded ideal microemulsion formulations (w/w)

Component (%)	M1	M2	M3
DPH HCl	2	2	2
Oleic acid	10.78	10.94	10.46
IPM	21.56	21.89	20.90
Transcutol HP	21.15	13.64	10.14
Tween 20	9.23	5.96	4.43
IPA	30.38	39.20	43.74
Distilled water	4.90	6.37	8.33

Characterization of microemulsion formulations

Viscosity

The viscosity of topical formulations represents a significant physical attribute affecting the rate of drug release. In general, increasing the viscosity of the formulation results in a hard structure and reduced drug release rate[34]. Contrarily, low viscosity plays a positive impact on the sprayability of formulations[35]. The viscosity results are presented in Table 4. For the blank formulations, viscosity values ranged between 5.74 and 7.92 mPa·s. Similarly, formulations incorporating DPH HCl demonstrated viscosities spanning from 5.96 to 8.30 mPa·s, offering a beneficial aspect for improved sprayability. Monton et al.[36] stated that the oral spray microemulsions they prepared using clove oil, tween 80, and PEG were suitable for administration with viscosity values between 12.8 and 65.8 mPa·s. However, the results indicated that the addition of the active ingredient to the formulations increased viscosity ($p<0.05$). In addition, consistent with the literature, it was detected that viscosity decreased as alcohol or water amounts increased in the microemulsion formulations[7,37,38] because of the low viscosities of these solvents.

Table 4. Characterization of the developed blank and DPH HCl-loaded microemulsion formulations

Formulation	Viscosity (mPa·s±SD)	Droplet size (nm±SD)	Turbidity (Ntu±SD)	PDI (±SD)	Refractive index (nD±SD)	Conductivity (µS/cm±SD)
M1	7.92±0.13	4.362±0.345	2.28±0.08	0.327±0.031	1.415±0.001	0.016±0.000
$M1_{DPH\ HCl}$	8.30±0.02	17.451±1.807	2.42±0.04	0.462±0.017	1.418±0.001	0.094±0.000
M2	6.43±0.08	5.501±0.658	2.08±0.03	0.326±0.028	1.410±0.001	0.011±0.000
$M2_{DPH\ HCl}$	6.82±0.17	19.030±0.707	2.50±0.04	0.516±0.029	1.414±0.001	0.127±0.001
M3	5.74±0.04	5.809±0.315	2.29±0.05	0.249±0.024	1.406±0.001	0.005±0.000
$M3_{DPH\ HCl}$	5.96±0.08	15.998±1.294	2.41±0.14	0.404±0.040	1.408±0.001	0.169±0.001

Droplet size, PDI, and conductivity

The droplet size of blank microemulsions was between 4.362 and 5.809 nm, while that of drug-loaded microemulsions was between 15.998 nm and 19.030 nm (Table 4). While microemulsions are generally characterized by droplet sizes of average 10-200 nm[39], smaller droplet sizes such as 1-10 nm can also be obtained[22,33,40,41]. These rather small droplet sizes can be attributed to the fact that the co-surfactant penetrates the interfacial film of the oil droplets and affects the fluidity and viscosity[20]. Additionally, the increase in droplet size may be associated with the addition of the active ingredient. Sarheed et al.[42] found an increase in droplet size in half of the formulations by adding lidocaine to blank nanoemulsions. The PDI of the microemulsions was found between 0.249 and 0.516 (Table 4). A PDI of less than 0.5 indicates that the formulations have narrow and homogeneous droplet size distribution[43].

Conductivity level is one of the key indicators for detecting the internal and external phases of the microemulsion. The presence of non-ionic surfactants and cosurfactants and low water content causes this value to be close to zero[22]. Additionally, since the oil phase mostly does not contain electrolytes, it causes low electrical conductivity values, and this may indicate that the external phase is oil[4,20]. The conductivity of both the drug-loaded and the blank microemulsions was found to be approximately zero (Table 4). Considering the obtained findings, it was determined that the formulations are W/O type microemulsions.

Turbidity, refractive index, and physical stability

Turbidity in a liquid formulation refers to blur in the formulation caused by suspended particles and particle size, which can affect both the formulation's

visual clarity and its physical stability[44,45]. An optimal microemulsion usually has minimal turbidity or high transparency, signifying a well-structured and stable formulation with finely dispersed and homogeneously mixed oil, water, and surfactant phases. The turbidity of the formulations was determined to range from 2.08 to 2.50 Ntu, as given in Table 4. The low turbidity values obtained indicated that the formulations were good in terms of physical appearance and stability. The results were consistent with the literature[46,47]. The refractive index is useful for characterizing and differentiating chemicals, evaluating their purity and clarity, and offering insight into the interactions and composition of pharmaceutical systems. The refractive index of all prepared microemulsions ranged between 1.408 and 1.418 (Table 4), and there was no significant difference in the presence and absence of DPH HCl although there was a very small increase with drug loading ($p>0.05$). This slight change indicated that drug loading was ensured without any phase change in the microemulsion, which was a complex system[4]. The physical stability of the formulations was demonstrated by the fact that no physical change was observed in drug-loaded and blank microemulsions subjected to challenging conditions such as temperature and centrifugation.

Sprayability

Sprayability is crucial for topical drug delivery as offers advantages such as the ability to deliver the formulation to difficult-to-apply areas and large areas for dermal application, short application time, and homogeneous distribution for colloidal preparations[48]. In addition, with all these advantages and ease of use, patient compliance is enhanced[49]. Therefore, the characterization of sprayability is necessary to evaluate the suitability of the formulation. The features and composition of the formulation, the size and shape of the nozzle, the pump's design, the reservoir's capacity, and the amount left in the spray bottle can impact the formulation's spray characteristics[50]. As shown in Figures 1a and 1b, to investigate the sprayability of the DPH HCl microemulsion, the formulations were sprayed to the horizontal plate using the texture analyzer. The spreading diameter of the formulations after one spray process was found to be between 5.150 and 6.240 mm (Table 5, Figure 1c). Due to the absence of leakage from the spray nozzle of the bottle and the large spray area, the sprayability of all tested formulations was evaluated as good. It was noted that the results were consistent with the viscosity, indicating that as viscosity decreased, the diameter of the spreading area increased[51].

Table 5. Diameter of spreading area of the drug-loaded formulations after one spray process (n=5)

Formulation	Diameter (cm±SD)
M1	5.150±0.137
M2	6.100±0.386
M3	6.240±0.185

In vitro drug release

The *in vitro* release study of the formulations was performed by the dialysis bag method. This method is a frequently preferred method for evaluating the release profiles of microemulsions[20,22]. DPH HCl release from the microemulsion formulations reached between 80% and 100% within 60 min (Figure 3). In a study, Aziz et al.[12] investigated the release profiles of DPH HCl-loaded chitosan nanoparticles by the dialysis bag method. It was observed that the drug reached 50-80% release levels within 60 min and 60-90% within 120 min. In our previous study[52], it was determined that the DPH HCl release from diatomite-chitosan composites was 60-90% within 60 min. The fact that the cumulative drug release reached 80-100% in a short time was consistent with the literature and this may be associated with the high water solubility of DPH HCl[52].

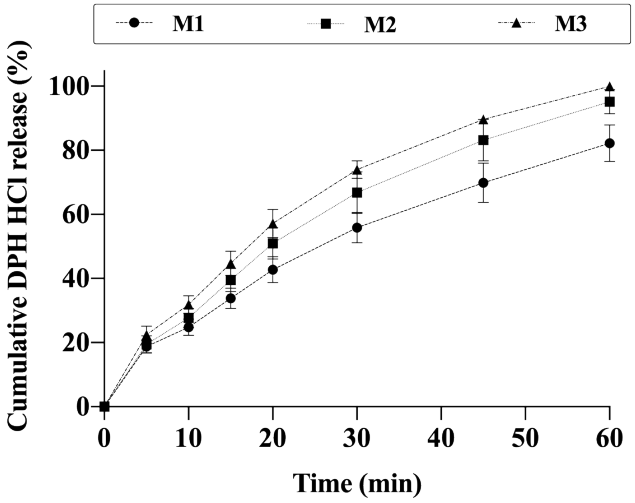

Figure 3. DPH HCl release profiles of the microemulsions (n=4)

The M3 formulation with the highest water content and the lowest viscosity provided higher drug release compared to others. Differences in the viscosities and the water content may have affected the drug release from the microemulsions. It is shown that there is generally a negative relationship between viscosity and drug release rate[41,53,54]. In a study, Kumari et al. developed the microemulsion formulations of valsartan and investigated the solubility of valsartan in microemulsion components. They also indicated the solubility of valsartan in the developed microemulsions. Among the microemulsions with no significant difference between their viscosities, M5 had the highest valsartan solubility and provided a higher cumulative release of valsartan compared to the other microemulsions[55]. The release profiles of the formulations were evaluated with model-dependent kinetics and it was found that the profiles were compatible with Higuchi kinetics, in which the highest r^2 value was determined (Table 6). This model suggests that drug release from microemulsions occurs through diffusion[22,56,57]. The similarity between the drug release profiles of the formulations was investigated by model-independent kinetics. The fact that the difference factor (f1) is less than 15 and the similarity factor (f2) is greater than 50 indicates the similarity of the drug release profiles of the formulations[25]. The release profiles of the M2 and M3 microemulsion pair were found to be similar (f1=10, f2=63) (Table 7).

Table 6. Release kinetics data of DPH HCl-loaded microemulsions

Formulation	Zero order (r^2)	First order (r^2)	Higuchi (r^2)	Hixson-Crowell (r^2)
M1	0.9711	0.8919	0.9909	0.9289
M2	0.9642	0.8578	0.9939	0.9021
M3	0.9486	0.8412	0.9898	0.8840

Table 7. Similarity and dissimilarity factors of the prepared formulations

Formulation pairs	Difference factor (f1)	Similarity factor (f2)
M1 – M2	17	52
M1 – M3	28	42
M2 – M3	10	63

Microemulsions are very convenient carriers for the penetration of active ingredients into the skin due to the penetration enhancers they contain, small droplet sizes, and the combination of oil and water. In addition to these advantages, their low viscosity allows them to be sprayed, providing ease of application and high patient compliance. In this study, a novel sprayable microemulsion formulation was designed for the dermal application of diphenhydramine hydrochloride, a first-generation antihistamine. The formulation with a surfactant/cosurfactant ratio of 1:3 showed the widest microemulsion region. The characterization studies of the obtained water/oil-type microemulsions have revealed their physical stability. The drug-loaded microemulsions (2%) with viscosity values of between 5.96 and 8.30 mPa·s were evaluated as having good sprayability with a great spreading area. *In vitro* drug release studies revealed that DPH HCl release from the microemulsions reached approx. 100% within 60 min and the best fit kinetic model for the DPH HCl release profiles was Higuchi, which suggests that the drug release occurs by diffusion. The findings indicated that these novel sprayable DPH HCl formulations are promising with high patient compliance for the topical treatment of itching-causing ailments such as insect bites, mild burns, and irritation.

STATEMENT OF ETHICS

Ethical approval was not required to perform this study.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Muhammet Davut Arpa: Supervision, Investigation, Project administration, Methodology, Writing – original draft, Writing - review and editing; Tuğba Arslan: Investigation, Methodology, Writing – original draft; Huriye Eraslan: Investigation, Methodology, Writing – original draft; Neslihan Üstündağ Okur: Supervision, Investigation, Writing - review and editing.

REFERENCES

1. Kaur L, Kumar R, Rahi DK, Sinha VR. Formulation and evaluation of microemulsion based gel of oriconazole for topical delivery. Anti-Infective Agents, 2017;15(2):95-104. Doi: 10.2174/2211352515666170619074437

2. Alkrad JA, Assaf SM, Hussein-Al-Ali SH, Alrousan R. Microemulsions as nanocarriers for oral and transdermal administration of enoxaparin. J Drug Deliv Sci Technol, 2022;70(103248):1-9. Doi: 10.1016/j.jddst.2022.103248

3. Szumała P, Macierzanka A. Topical delivery of pharmaceutical and cosmetic macromolecules using microemulsion systems. Int J Pharm, 2022;615(121488):1-12. Doi: 10.1016/j.ijpharm.2022.121488

4. Lamoudi L, Akretche S, Hadjsadok A, Daoud K. Fusidic acid microemulsion based on a pseudoternary phase diagram: development, characterization, and evaluation. J Pharm Innov, 2022. Doi: 10.1007/s12247-022-09668-4

5. Hajjar B, Zier KI, Khalid N, Azarmi S, Löbenberg R. Evaluation of a microemulsion-based gel formulation for topical drug delivery of diclofenac sodium. J Pharm Investig, 2018;48(3):351-362. Doi: 10.1007/s40005-017-0327-7

6. Muzaffar F, Singh UK, Chauhan L. Review on microemulsion as futuristic drug delivery. Int J Pharm Pharm Sci, 2013;5(3):39-53.

7. Üstündağ Okur N, Çağlar EŞ, Arpa MD, Karasulu HY. Preparation and evaluation of novel microemulsion-based hydrogels for dermal delivery of benzocaine. Pharm Dev Technol, 2017;22(4):500-510. Doi: 10.3109/10837450.2015.1131716

8. Zhang Q, Jiang X, Jiang W, Lu W, Su L, Shi Z. Preparation of nimodipine-loaded microemulsion for intranasal delivery and evaluation on the targeting efficiency to the brain. Int J Pharm, 2004:275:85-96. Doi: 10.1016/j.ijpharm.2004.01.039

9. Miller SM, Cumpston KL. Diphenhydramine. Philip Wexler (Ed.) In Encycl Toxicol (Third Ed), 2014;2:195-197. Doi: 10.1016/B978-0-12-386454-3.00724-7

10. Gumieniczek A, Lejwoda K, Data N. Chemical stability study of H(1) antihistaminic drugs from the first and the second generations, diphenhydramine, azelastine and bepotastine, in pure APIs and in the presence of two excipients, citric acid and polyvinyl alcohol. Molecules, 2022;27(23):8322. Doi: 10.3390/molecules27238322

11. Baby A, Shivakumar HN, Alayadan P. Formulation and evaluation of film forming solution of diphenhydramine hydrochloride for transdermal delivery. Indian J Pharm Educ Res, 2022;56(1):43-49. Doi: 10.5530/ijper.56.1.6

12. Aziz SN, Badawy AA, Nessem DI, Abd El Malak NS. Promising nanoparticulate system for topical delivery of diphenhydramine hydrochloride: in-vitro and in-vivo evaluation. J Drug Deliv Sci Technol, 2020;55:101454. Doi: 10.1016/j.jddst.2019.101454

13. Pavlidakey PG, Brodell EE, Helms SE. Diphenhydramine as an alternative local anesthetic agent. J Clin Aesthet Dermatol, 2009;2(10):37-40.

14. Neubert RHH, Schmalfuß U, Wolf R, Wohlrab WA. Microemulsions as Colloidal Vehicle Systems for Dermal Drug Delivery. Part V: Microemulsions without and with Glycolipid as Penetration Enhancer. J Pharm Sci, 2005;94(4):821-827. Doi: 10.1002/jps.20233

15. Sanna V, Peana AT, Moretti MDL. Development of new topical formulations of diphenhydramine hydrochloride: In vitro diffusion and in vivo preliminary studies. Int J PharmTech Res, 2010;2(1):863-869.

16. Kotwal V, Bhise K, Thube R. Enhancement of iontophoretic transport of diphenhydramine hydrochloride thermosensitive gel by optimization of pH, polymer concentration, electrode design, and pulse rate. AAPS PharmSciTech, 2007;8(4):E1-E6. Doi: 10.1208/pt0804120

17. Schmalfuß U, Neubert R, Wohlrab W. Modification of drug penetration into human skin using microemulsions. J Control Release, 1997;46(3):279-285. Doi: 10.1016/S0168-3659(96)01609-4

18. Arpa MD, Ünükür MZ, Erim ÜC. Formulation, characterization and in vitro release studies of terbinafine hydrochloride loaded buccal films. J Res Pharm, 2021;25(5):667-680. Doi: 10.29228/jrp.58

19. Berkman M, Güleç K. Pseudo Ternary Phase Diagrams: a practical approach for the area and centroid calculation of stable microemulsion regions. Istanbul J Pharm, 2021;51(1):42-49. Doi: 10.26650/istanbuljpharm.2020.0090

20. Yozgatlı V, Üstündağ Okur N, Okur ME, Sipahi H, Charehsaz M, Aydın A, et al. Managing allergic conjunctivitis via ophthalmic microemulsions: Formulation, characterization, in vitro irritation studies based on EpiOcular™ eye irritation assay and in vivo studies in rabbit eye. J Surfactants Deterg, 2023;26(6):853-866. Doi: 10.1002/jsde.12691

21. Okur ME, Kolbaşı B, Şahin M, Karadağ AE, Reis R, Çağlar EŞ, et al. A novel approach in the treatment of osteoarthritis: in vitro and in vivo evaluation of *Allium sativum* microemulsion. J Surfactants Deterg, 2022;25(5):621-633. Doi: 10.1002/jsde.12605

22. Çağlar EŞ, Okur ME, Aksu B, Üstündağ Okur N. Transdermal delivery of acemetacin loaded microemulsions: Preparation, characterization, in vitro-ex vivo evaluation and in vivo analgesic and anti-inflammatory efficacy. J Dispers Sci Technol, 2023:1-11. Doi: 10.1080/01932691.2023.2175691

23. Karasulu HY, Oruç N, Üstündağ Okur N, Ilem Özdemir D, Ay Şenyiğit Z, Barbet Yilmaz F, et al. Aprotinin revisited: formulation, characterization, biodistribution and therapeutic potential of new aprotinin microemulsion in acute pancreatitis. J Drug Target, 2015;23(6):525-537. Doi: 10.3109/1061186X.2015.1015537

24. Arpa MD, Kesmen EE, Biltekin SN. Novel sprayable thermosensitive benzydamine hydrogels for topical application: development, characterization, and in vitro biological activities. AAPS PharmSciTech, 2023;24(8):1-16. Doi: 10.1208/s12249-023-02674-w

25. Arpa MD, Üstündağ Okur N, Gök MK, Özgümüş S, Cevher E. Chitosan-based buccal mucoadhesive patches to enhance the systemic bioavailability of tizanidine. Int J Pharm, 2023;642(123168):1-13. Doi: 10.1016/j.ijpharm.2023.123168

26. Sahoo S, Pani NR, Sahoo SK. Microemulsion based topical hydrogel of sertaconazole: formulation, characterization and evaluation. Colloids Surfaces B Biointerfaces, 2014;120:193-199. Doi: 10.1016/j.colsurfb.2014.05.022

27. Yang, J, Xu H, Wu S, Ju B, Zhu D, Yan Y, et al. Preparation and evaluation of microemulsion-based transdermal delivery of *Cistanche tubulosa* Phenylethanoid Glycosides. Mol Med Rep, 2017;15(3):1109-1116. Doi: 10.3892/mmr.2017.6147

28. Moghimipour E, Farsimadan N, Salimi A. Ocular delivery of quercetin using microemulsion system: design, characterization, and ex-vivo transcorneal permeation. Iran J Pharm Res, 2022;21(1):e127486. Doi: 10.5812/ijpr-127486

29. Malakar J, Sen SO, Nayak AK, Sen KK. Development and evaluation of microemulsions for transdermal delivery of insulin. ISRN Pharm, 2011;780150:1-7. Doi: 10.5402/2011/780150

30. Ita K. Chemical Permeation Enhancers. Ita K (Ed.). In Transdermal Drug Delivery, Academic Press,2020:p. 63-96. Doi: 10.1016/b978-0-12-822550-9.00005-3

31. Hernandez C, Jain P, Sharma H, Lam S, Sonti S. Investigating the effect of transcutol on the physical properties of an O/W cream. J Dispers Sci Technol, 2020;41(4):600-606. Doi: 10.1080/01932691.2019.1609362

32. Schmidt RF, Prause A, Prévost S, Doutch J, Gradzielski M. Phase behavior and structure of a biocompatible microemulsion based on Tween 20, 2-ethylhexylglycerine and isopropyl palmitate in water. Colloid Polym Sci, 2023;301(7):753-762. Doi: 10.1007/s00396-023-05119-9

33. Bergonzi MC, Hamdouch R, Mazzacuva F, Isacchi B, Bilia AR. Optimization, characterization and in vitro evaluation of curcumin microemulsions. LWT-Food Sci Technol, 2014;59(1):148-155. Doi: 10.1016/j.lwt.2014.06.009

34. Binder L, Mazál J, Petz R, Klang V, Valenta C. The role of viscosity on skin penetration from cellulose ether-based hydrogels. Ski Res Technol, 2019;25(5):725-734. Doi: 10.1111/srt.12709

35. Mahdi MH, Conway BR, Smith AM. Development of mucoadhesive sprayable gellan gum fluid gels. Int J Pharm, 2015;488(1-2):12-19. Doi: 10.1016/j.ijpharm.2015.04.011

36. Monton C, Settharaksa S, Suksaeree J, Chusut T. The preparation, characterization, and stability evaluation of a microemulsion-based oral spray containing clove oil for the treatment of oral candidiasis. J Drug Deliv Sci Technol, 2020;57:101735. Doi: 10.1016/j.jddst.2020.101735

37. Manyala DL, Varade D. Formation and characterization of microemulsion with novel anionic sodium n-lauroylsarcosinate for personal care. J Mol Liq, 2021;343:117657. Doi: 10.1016/j.molliq.2021.117657

38. Sharma H, Kumar Sahu G, Kaur CD. Development of ionic liquid microemulsion for transdermal delivery of a chemotherapeutic agent. SN Appl Sci, 2021;3(2). Doi: 10.1007/s42452-021-04235-x

39. Bhalke RD, Kulkarni SS, Kendre PN, Pande VV, Giri MA. A facile approach to fabrication and characterization of novel herbal microemulsion-based UV shielding cream. Futur J Pharm Sci, 2020;6(1):1-10. Doi: 10.1186/s43094-020-00075-5

40. Moghimipour E, Salimi A, Eftekhari S. Design and characterization of microemulsion systems for naproxen. Adv Pharm Bull, 2013;3(1):63-71. Doi: 10.5681/apb.2013.011

41. Salimi A, Noorafrooz R, Fouladi M, Soleymani, SM. Design and assessment of a microemulsion-based transdermal drug delivery system for meloxicam; examination of formulation ingredients. Fabad J Pharm Sci, 2023;48(3):359-372. Doi: 10.55262/fabadeczacilik.1187620

42. Sarheed O, Dibi M, Ramesh KVRNS. Studies on the effect of oil and surfactant on the formation of alginate-based O/W lidocaine nanocarriers using nanoemulsion template. Pharmaceutics, 2020;12(12):1-21. Doi: 10.3390/pharmaceutics12121223

43. Alnusaire TS. Olive leaves (*Olea europaea* L.) extract loaded lipid nanoparticles: optimization of processing parameters by box-behnken statistical design, *in-vitro* characterization, and evaluation of anti-oxadant and anti-microbial activity. J Oleo Sci, 2021;70(10):1403-1416. Doi: 10.5650/jos.ess21149

44. Ningrum YP, Budhiyanti SA. Formulation and stability of *Ulva lactuca* fatty acid oil in water (O/W) microemulsion. Food Research, 2022;6(4):120-127. Doi: 10.26656/fr.2017.6(4).575

45. Cho YH, Kim S, Bae EK, Mok CK, Park J. Formulation of a cosurfactant-free O/W microemulsion using nonionic surfactant mixtures. J Food Sci, 2008;73(3):115-121. Doi: 10.1111/j.1750-3841.2008.00688.x

46. Çilek A, Çelebi N, Tirnaksiz F. Lecithin-based microemulsion of a peptide for oral administration: preparation, characterization, and physical stability of the formulation. Drug Deliv, 2006;13(1):19-24. Doi: 10.1080/10717540500313109

47. Xiao L, Hou J, Wang W, Raj I. Development of a novel high-temperature microemulsion for enhanced oil recovery in tight oil reservoirs. Materials, 2023;16(19):6613. Doi: 10.3390/ma16196613

48. Pleguezuelos-Beltrán P, Gálvez-Martín P, Nieto-García D, Marchal JA, López-Ruiz E. Advances in spray products for skin regeneration. Bioact Mater, 2022;16:187-203. Doi: 10.1016/j.bioactmat.2022.02.023

49. Umar AK, Butarbutar M, Sriwidodo S, Wathoni N. Film-forming sprays for topical drug delivery. Drug Des Devel Ther, 2020;14:2909-2925. Doi: 10.2147/DDDT.S256666

50. Gholizadeh H, Messerotti E, Pozzoli M, Cheng S, Traini D, Young P, et al. Application of a thermosensitive in situ gel of chitosan-based nasal spray loaded with tranexamic acid for localised treatment of nasal wounds. AAPS PharmSciTech, 2019;20(7):1-12. Doi: 10.1208/s12249-019-1517-6

51. Prüfert F, Hering U, Zaichik S, Le NMN, Bernkop-Schnürch A. Synthesis and in vitro characterization of a preactivated thiolated acrylic acid/acrylamide-methylpropane sulfonic acid copolymer as a mucoadhesive sprayable polymer. Int J Pharm, 2020,583:119371. Doi: 10.1016/j.ijpharm.2020.119371

52. Özkan Z, Arpa MD, Özçatal M, Çiftçi H. Preparation of Diatomite-Chitosan Composites for Loading and Release of Diphenhydramine HCl. JournalMM, 2023;4(2):542-560. Doi: 10.55546/jmm.1326482

53. Mali KK, Dhawale SC, Dias RJ. Microemulsion based bioadhesive gel of itraconazole using tamarind gum: in-vitro and ex-vivo evaluation. Marmara Pharm J, 2017;21(3):688-688. Doi: 10.12991/marupj.323593

54. Kamila MM, Mondal N, Gupta BK, Ghosh LK. Preparation, characterization and in-vitro evaluation of sunflower oil-Tween 80-glycerol-based microemulsion formulation of a BCS class-II drug. Lat Am J Pharm, 2009;28(4):622-627.

55. Kumari P, Kumar K, Joshi A, Chauhan V, Rajput V. Development and evaluation of microemulsion Formualatons of Valsartan for Solubility Enhancement. J Drug Deliv Ther, 2023;13(10):117-120. Doi: 10.22270/jddt.v13i10.5990

56. Shewaiter MA, Hammady TM, El-Gindy A, Hammadi SH, Gad S. Formulation and characterization of leflunomide/diclofenac sodium microemulsion base-gel for the transdermal treatment of inflammatory joint diseases. J Drug Deliv Sci Technol, 2021;61:102110. Doi: 10.1016/j.jddst.2020.102110

57. De Stefani C, Vasarri M, Salvatici MC, Grifoni L, Quintela JC, Bilia AR, et al. Microemulsions enhance the in vitro antioxidant activity of oleanolic acid in RAW 264.7 Cells, Pharmaceutics, 2022;14(10):2232. Doi: 10.3390/pharmaceutics14102232