Stability of Extemporaneously Prepared Amitriptyline Hydrochloride Topical Preparations for the Treatment of Neuropathic Pain

Piyanuch Rojsanga, Ph.D.*, Anchalee Jintapattanakit, Ph.D., Doungdaw Chantasart, Ph.D.****

**Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand, **Department of Pharmacy, Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand.*

ABSTRACT

Objective: The aim of this study was to investigate the physicochemical and microbiological stability of extemporaneous amitriptyline hydrochloride (AMH) topical preparations for the treatment of neuropathic pain.

Materials and Methods: AMH tablets were triturated to produce fine powders with a mortar and pestle. These powders were levigated and separately incorporated into four compounding bases: hydrophilic petrolatum USP, anionic cream, cold cream USP, and pluronic lecithin organogel (PLO) having the concentration of 2%w/w AMH.

Results: In the *in vitro* release study, the most significant amount of AMH was released from the PLO, followed by cold cream, anionic cream and hydrophilic petrolatum, respectively; therefore, the compounded AMH in cold cream and AMH in PLO were selected for the evaluation of the *in vitro* permeation and product stability. The permeation of AMH from PLO across human epidermal membrane was significantly greater than that from the cold cream. Product stability was characterized as having no remarkable change in color or texture and AMH remaining in the range of 90–110% of the initial concentration quantified by high-performance liquid chromatography. Compounded AMH in cold cream was stable at 2–8 °C and 30 °C for 60 days, and 40 °C for 30 days, whereas compounded AMH in PLO was stable at 30 °C and 40 °C for 14 days. There was no visible microbial growth in any of the samples.

Conclusion: Taken together with the *in vitro* permeation and product stability studies, the present study suggests that AMH in cold cream could be prepared and used as extemporaneous topical preparations with a beyond-use date of 60 days when kept at 2–8 °C and 30 °C.

Keywords: Amitriptyline hydrochloride; neuropathic pain; cold cream; pluronic lecithin organogel; extemporaneous topical preparations (Siriraj Med J 2023; 75: 427-435)

INTRODUCTION

Chronic pain is usually caused by an initial trauma, such as a back sprain or muscle strain.[1] It is believed that chronic pain develops after nerves become impaired. Chronic pain is associated with a diminished quality of life, increased health expenditure, and high economic costs. It persists for months or years and can interfere with daily life activities, such as working, having a social life, and taking care of oneself and others. It has been estimated that chronic pain affects 10 percent of the world's population.[2] However, the phrase "neuropathic pain" which reflects both peripheral and central sensitization mechanisms, came into common use only in the last decade. Important pathophysiologic mechanisms of neuropathic pain are sodium-and calcium-channel upregulation, spinal hyperexcitability, descending facilitation, and aberrant sympathetic-somatic nervous system interactions.[3] Current recommended first-line

Corresponding author: Doungdaw Chantasart
E-mail: doungdaw.cha@mahidol.ac.th
Received 3 March 2023 Revised 21 April 2023 Accepted 1 May 2023
ORCID ID:http://orcid.org/0009-0008-2231-0575
https://doi.org/10.33192/smj.v75i6.261621

treatments include antidepressants (tricyclic agents and serotonin-norepinephrine reuptake inhibitors) and anticonvulsants (gabapentin and pregabalin).[3]

Amitriptyline is a dibenzocycloheptene-derivative tricyclic antidepressant agent that acts upon many sites. It can act within the central nervous system by inhibiting neuronal reuptake of norepinephrine and serotonin. Moreover, amitriptyline can act within the periphery by blocking Na^+, K^+, and Ca^{2+} voltage-gated ion channels and various receptors (muscarinic, cholinergic, nicotinic, histaminergic, α_2-adrenergic, adenosine, and N-methyl-D-aspartate receptors).[4-6] Amitriptyline, administered orally, is currently one of the treatment options available for managing neuropathic pain. However, the following adverse effects have been reported for oral administration of amitriptyline: drowsiness, dizziness, dry mouth, constipation and sweating.[7] Nowadays, topical treatments for managing peripheral neuropathic pain are gaining popularity due to the excellent safety profiles and preferences. Due to its physicochemical properties (MW 277.4 g/mole and log P 4.92),[8] amitriptyline is a promising candidate for delivery as a topical analgesic. In a recent review, topically applied amitriptyline at concentrations between 2% and 10% were successfully used for clinical neuropathic pain treatment.[9-12] Thompson and Brooks[13] found that patients who received higher concentration of amitriptyline experienced greater pain relief, but there were also more reports of adverse effects, including systemic absorption and skin irritation at the site of application. To avoid these adverse effects, a topical preparation of 2% amitriptyline hydrochloride (AMH) was chosen. However, the information regarding the types of compounding bases and the formulations is limited. In addition, there are no marketed topical amitriptyline products currently available. In this study, AMH compounded with four compounding bases (i.e., hydrophilic petrolatum USP, anionic cream, cold cream USP, and pluronic lecithin organogel (PLO)) were prepared. The *in vitro* drug release was carried out in order to identify the formulations with suitable drug release profiles. Furthermore, *in vitro* skin permeation and stability studies were conducted to evaluate their potential for use as extemporaneous topical preparations for the treatment of neuropathic pain.

MATERIALS AND METHODS

AMH (≥98% purity analyzed by thin layer chromatography) was purchased from Sigma-Aldrich (St. Louis, MO, USA). The commercial AMH tablets (containing 25 mg per tablet) were kindly provided by the Government Pharmaceutical Organization, Thailand. Tryptic soya agar (TSA) and sabouraud dextrose agar (SDA) were supplied by Becton, Dickinson and Company (Franklin Lakes, New Jersey). High-performance liquid chromatography (HPLC) grade methanol and acetonitrile were supplied by Honeywell Burdick and Jackson (Ulsan, Korea). All the other materials and solvents used were of analytical reagent grade.

Human abdominal skin (HEM) of female patients aged 30 – 60 years was obtained from abdominoplastic surgical operations (Department of Surgery, Yanhee General Hospital, Thailand). HEM, which includes the stratum corneum and viable epidermis, was separated from the dermis by heat separation technique as described by Chantasart et al.[14] The use of human tissue was reviewed and approved by the committee on human rights related to human experimentation, Mahidol University, Thailand (COE. No. MU-DT/PY-IRB 2016/014.0208).

Preparation of AMH formulations

Four compounding bases (i.e., hydrophilic petrolatum USP, anionic cream, cold cream USP, and PLO) were prepared in accordance with the art, science, and technology of pharmaceutical compounding.[15] These compounding bases were selected because of their frequency of use in many topical formulations. In addition, hydrophilic petrolatum, anionic cream, and cold cream are official USP ointment bases, and it has been reported that PLO promotes the release of hydrophobic drugs.[16-19] Therefore, they were selected as the compounding bases in this study. The compositions and preparation of the four compounding bases are provided in the Supplementary Materials.

To prepare 2%w/w AMH in each compounding base, an appropriate amount of AMH tablets were weighed and triturated to produce fine powders with a mortar and pestle. The powders were levigated and incorporated separately into each of the compounding bases. Mineral oil was the levigating agent used with the incorporation of the drug powders into the hydrophilic petrolatum and cold cream, whereas purified water was the levigating agent used with the drug powders in the anionic cream and PLO. The required levigating agent was introduced to the fine powders in order to achieve a smooth paste. Each compounding base was then added to the prepared paste using the geometric dilution method. All AMH preparations were packed into plastic containers, as shown in Fig 1.

HPLC analysis for AMH

Experiments were performed on a Shimadzu LC-10A system (Shimadzu, Kyoto, Japan) equipped with a model

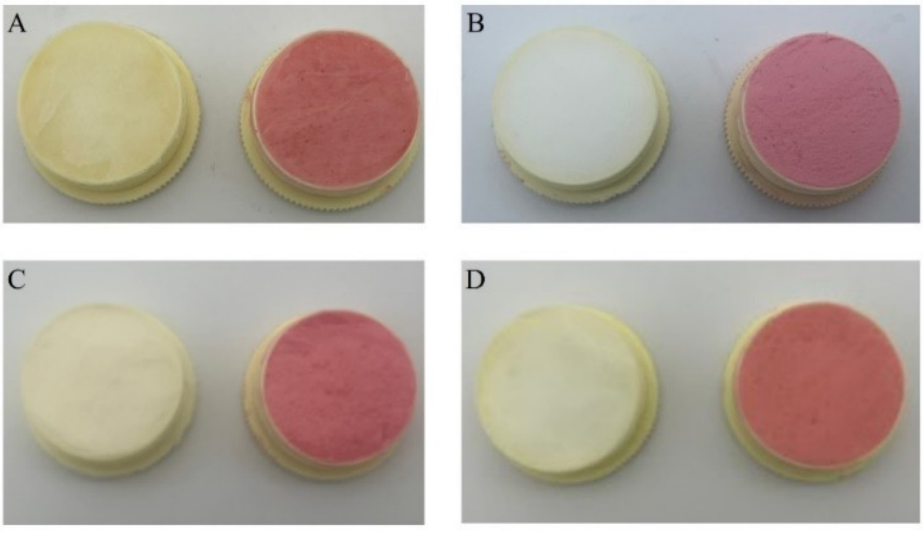

Fig 1. Appearance of the formulations in various compounding bases, which are (A) hydrophilic petrolatum USP, (B) anionic cream, (C) cold cream USP, and (D) PLO. White or yellowish color represents compounding bases (without drug). Pink color creams represent bases that 2% AMH was incorporated.

series LC-10AD pump, CBM-10A system controller, DGU-12 A degasser and an SPD-10A diode array detector with C8 column (5 µm, 150 mm×4.6 mm) (GL Sciences, Netherlands). The mobile phase consisted of a phosphoric acid, pH 2.0, and acetonitrile (60:40, v/v). The column temperature and flow rate were kept at 25 °C and 1 mL/min, respectively. The diode array detector was set at 240 nm, and the injection volume was 50 µL.

Sample preparation

On the day of the analysis, the samples were placed at room temperature for 1 h, and 200 mg of each formulation (equivalent to 4 mg of AMH) was mixed with 10 mL of methanol in a 15-mL centrifuge tube. The mixture was then heated at 80 °C in a water bath for 10 min with occasional shaking. Each tube was then removed from the bath, sonicated for 10 min, followed by 5 min of vigorously shaking, before being promptly centrifuged. The supernatant was then transferred to a 100-mL volumetric flask. The extraction procedure was repeated twice, using 10 mL of methanol in the centrifuge tube each time. The combined supernatant in the volumetric flask was diluted with methanol to reach the desired volume, mixed thoroughly volume, mixed, and filtered with a polytetrafluoroethylene membrane of 0.45 µm before injection. The % of AMH remaining in each formulation was calculated using the calibration curve.

Method validation

The chromatographic method was validated for specificity, linearity, range, precision, and accuracy according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use guideline.[20] Forced degradation studies on AMH in cold cream were carried out to investigate the specificity of the method. The AMH in the cold cream base was exposed to water, 0.05 N hydrochloric acid, 0.05 N sodium hydroxide, and 0.3%w/v hydrogen peroxide at 80 °C for 2 h. The samples were then extracted as described in the sample preparation. The selectivity of the method was achieved as the peak purity of the AMH peak was more than 0.95. The method's linearity was tested using AMH concentrations ranging from 10 to 100 µg/mL, and the linear regression and correlation coefficient (r) were calculated. Accuracy was measured by standard addition at three different concentrations of AMH (10, 20, and 40 µg/mL) to the AMH in cold cream. Then, the % recovery of AMH was calculated. The intra-day precision or repeatability of the method was estimated by calculating the relative standard deviation (%RSD) of a sample spiked with three concentrations of standard AMH from the recovery study (n = 9) on two different days and analysis of intermediated precision (n = 18).

For the *in vitro* skin permeation study, specificity was assessed by comparing the chromatograms of AMH in the receptor medium alone and each sample solution from receiving chamber of the cold cream and PLO. The linearity of the method was in the range of 0.63-10 µg/mL. Accuracy was evaluated by standard addition at three different concentrations of AMH (1.25, 2.5, 5 µg/mL) to each sample solution obtained from the cold cream and PLO's donor chamber. Precision was evaluated by analyzing the sample solutions at 8 µg/mL (n = 6) on the same day for repeatability and on two different days as well as analysis for intermediated precision (n = 12). Finally, the %RSD was calculated.

***In vitro* drug release study**

The *in vitro* release of AMH from four formulations was studied using Franz diffusion cells with an effective area of ~2.40 cm^2. Each formulation was spread onto a

regenerated cellulose membrane (Spectra/Por®4 MWCO 12,000–14,000, Spectrum Laboratories, Inc., Rancho Dominguez, California) that had been treated with citrate buffer pH 5.5. The receiver chamber was filled with a precise amount of degassed citrate buffer pH 5.5 (~10 mL) and continuously stirred with a magnetic stir bar. The temperature of receiver solution was maintained at 32 ± 1°C. An aliquot of 300 µL receiver fluid was collected at specified time intervals (i.e., 0.25, 0.5, 1, 2, 4, 6, 8, 12, and 24 h) and immediately replaced in the chamber with the same volume of citrate buffer pH 5.5 to maintain a constant volume. The AMH content in the collected sample was analyzed by HPLC. Data are expressed as the cumulative amount of AMH release per surface area (µg/cm^2).

In vitro skin permeation study

The *in vitro* drug permeation of AMH from AMH in cold cream and PLO across HEM was studied using Franz diffusion cells. HEM (~4x4 cm^2) was mounted on the diffusion cells with an effective area of ~2.40 cm^2. A regenerated cellulose membrane was placed between the viable epidermis side of the HEM sample and the receiver chamber.[14,21] Each formulation was spread onto the epidermis side of the HEM. The receiver chamber was filled with a precise amount of degassed citrate buffer pH 5.5 (~10 mL) and continuously stirred with a magnetic stir bar. The temperature of receiver was maintained at 32 ± 1 °C. An aliquot of 300 µL solutions were collected from the receiver chambers at specific time intervals (4, 6, 8, 10, 12, and 24 h), and then replaced with the same volume of fresh receptor media. The AMH content in the collected sample was analyzed by HPLC. Data are expressed as the cumulative amount of AMH permeation per surface area (µg/cm^2).

Stability study

In order to study the stability of the AMH formulations, AMH in cold cream was studied under three conditions, including in refrigerator (2–8 °C), room temperature (30 °C), and an accelerated condition (40 °C). Poloxamer 407, a thermosensitive gel forming agent, changes to a fluid state at 2–8 °C; therefore, the stability of AMH in PLO was studied under two conditions, including 30 °C and 40 °C.

Physical stability testing

The physical characteristics of AMH in cold cream and in PLO were investigated. The physical appearance, color, homogeneity, phase separation, and texture were monitored at each sampling time point. The formulation viscosity was measured using a rheometer (HAAKE RotoVisco 1 Rotational Rheometer, Therma Fisher Scientific, Germany) with a cone and plate (35/2° Ti) model at 5.00 s^{-1} shear rate at 30 °C for 5 min. The sample tests were conducted on days 0, 14, 30 and 60.

Chemical stability testing

Chemical stability testing was conducted with a quantitative analysis of AMH using HPLC. The sample tests were conducted on days 0, 14, 30, and 60.

Microbiological stability testing

The AMH in cold cream and in PLO kept under room temperature were tested for their microbiological specifications following the United States Pharmacopeia 41 (USP41) Chapter <61> Microbiological Examination of Nonsterile Products: Microbial Enumeration Test.[22] The total aerobic microbial count (TAMC) was conducted using the pour plate method with TSA incubated at 30 °C to 35 °C for three days. In addition, the total combined yeasts and molds count (TYMC) was conducted using the pour plate method with SDA incubated at 20 °C to 25 °C for five days.

Data analysis

Data are expressed as mean ± SD, and statistical significance was determined using repeated measures and the Two-way ANOVA multiple comparisons test. All statistical tests were performed using GraphPad Prism version 7.00 for Windows (GraphPad Software, California). A *p*-value of less than 0.05 was considered significant.

Stability was defined as no dramatic changes in appearance, color or viscosity. Moreover, the initial AMH concentration (day 0) analyzed by HPLC was defined as 100%, and the subsequent concentrations of each time point were calculated as percentages of the initial concentration. According to the USP, the acceptable limit for most compounded pharmaceutical preparations is typically ± 10%, or within the range of 90.0% to 110.0 % of the active pharmaceutical ingredient.[23,24]

RESULTS AND DISCUSSION

HPLC method validation

The analysis of the cold cream base and forced degradation samples prepared in the same manner as the samples under the proposed chromatographic conditions showed no interference with the AMH peak, indicating the specificity of the method. Forced degradation is the degradation of AMH in cold cream in conditions that are more severe than accelerated conditions, which is

necessary to demonstrate the specificity of the methods to determine stability. Fig 2 shows the chromatograms of AMH compounded in cold cream. The various stability behaviors of AMH were observed when subjected to neutral (Fig 2B), acidic (Fig 2C), basic (Fig 2D), and oxidizing (Fig 2E) conditions. Although AMH in cold cream remained chemically stable when heated at 80 °C for 2 h (neutral hydrolysis), it degraded under the acidic, basic, and oxidizing conditions at a rate of 22.2%, 27.8% and 47.5%, respectively. The fact that the degradation peaks and AMH were separated under our chromatographic conditions with a peak purity of more than 0.95 indicated the reliability of the assay for stability evaluation.

For the HPLC analysis in the skin permeation study, the chromatograms of AMH in the receptor medium and the samples from the receiving chamber of either the cold cream base or PLO base showed no interference with regard to the AMH peak, revealing the specificity of the method. As shown in Table 1, HPLC method was found to be linear, accurate and precise for the assay of AMH in the formulations and skin permeation study.

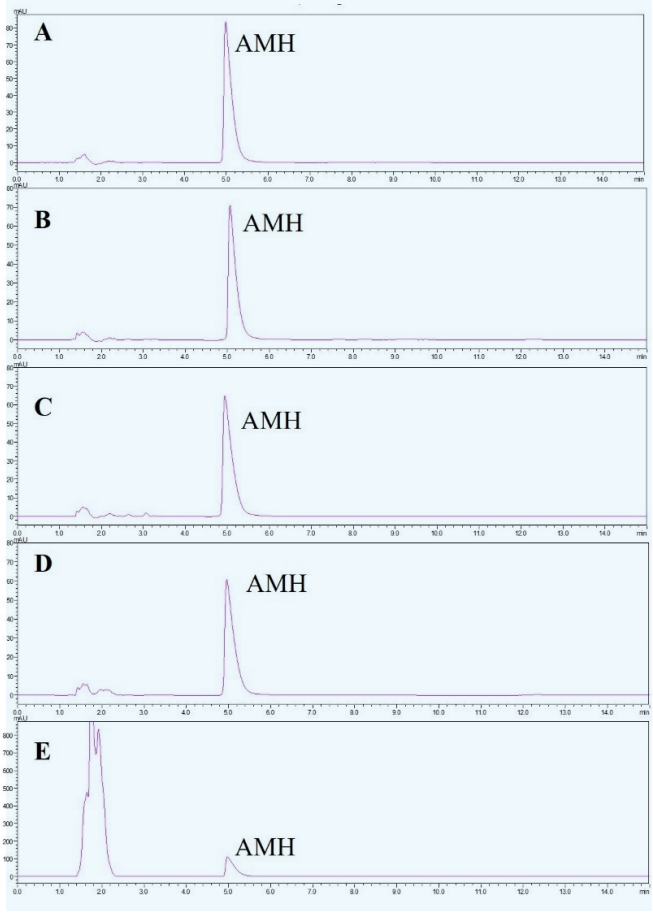

Fig 2. Chromatograms of AMH compounded in cold cream under various conditions
(A)control, (B) heat condition, (C) acidic condition, (D) basic condition, and (E) oxidizing condition

Preparation of extemporaneous AMH topical formulations

Four separate AMH topical formulations were prepared (Fig 1). Each formulation had a pink color and good spreadability. However, the AMH in hydrophilic petrolatum and the AMH in cold cream had a greasy texture and were difficult to wash out with water, whereas the AMH in anionic cream and AMH in PLO had a light non-greasy texture and were easy to wash out with water.

***In vitro* drug release study**

Because the drugs need to be released from the topical base before permeating through the skin, *in vitro* drug release tests from drug preparations across cellulose membranes are often performed before skin permeation studies.[25] It is possible to determine how the physicochemical properties of the topical bases and the ingredients affect each formulation's drug release profiles. The release profiles of AMH from the various topical bases over 24 h are shown in Fig 3. It is clearly illustrated that AMH exhibits the highest drug release performances from the PLO (5,025 ± 682 μg/cm^2), followed by cold cream (1,609 ± 299 μg/cm^2), anionic cream (484 ± 256 μg/cm^2) and hydrophilic petrolatum (134 ± 33 μg/cm^2), respectively. The results obtained could be explained by AMH's physicochemical properties and the formulations of the topical vehicles.

Based on the fact that electrolytes dissociate in ion forms when dissolved in water, in the topical base containing water, AMH, the salt form of the weak base would dissociate into amitriptyline and hydrochloride. Consequently, amitriptyline is dissolved in an oil phase. Several studies have reported that the PLO promotes the release of hydrophobic drugs.[16-19] Together with the weak affinity between the poloxamer and amitriptyline, the coexistence of organic and aqueous phases through the structurally well-defined micellar network of phospholipids of PLO, in which low water-soluble amitriptyline can be entrapped within the gel matrix,[26] may facilitate the release of amitriptyline (expressed as AMH) from PLO.[19]

The presence of surfactants in the system can impact the solubility of the hydrophobic drug and improve the drug release rate. The hydrophobic nature of the external environment of a w/o emulsion facilitates the release of the hydrophobic drug from the external oil phase.[27] Therefore, the release of amitriptyline from cold cream would be faster than that from anionic cream (o/w emulsion). In the case of hydrophobic petrolatum, an absorption base, that contains the water-absorbing material of white wax, the ointment matrix was completely immiscible with water and the AMH was dispersed in the base.

TABLE 1. HPLC method validation data of AMH.

Validation parameter		Assay	Skin permeation study
Linearity		y = 43885x - 83043 r = 0.9997	y = 27948x + 283.54 r = 0.9999
Range		10 - 100 µg/mL	6.25 - 10 µg/mL
Accuracy	Cold cream	95.13 - 101.69 %	97.01 - 102.03%
	PLO	95.99 - 102.36%	94.87 - 101.28%
Repeatability (%RSD)	Cold cream	< 2.14	< 1.20
	PLO	< 1.98	< 0.10
Intermediate Precision (%RSD)	Cold cream	2.09	1.79
	PLO	1.74	0.13

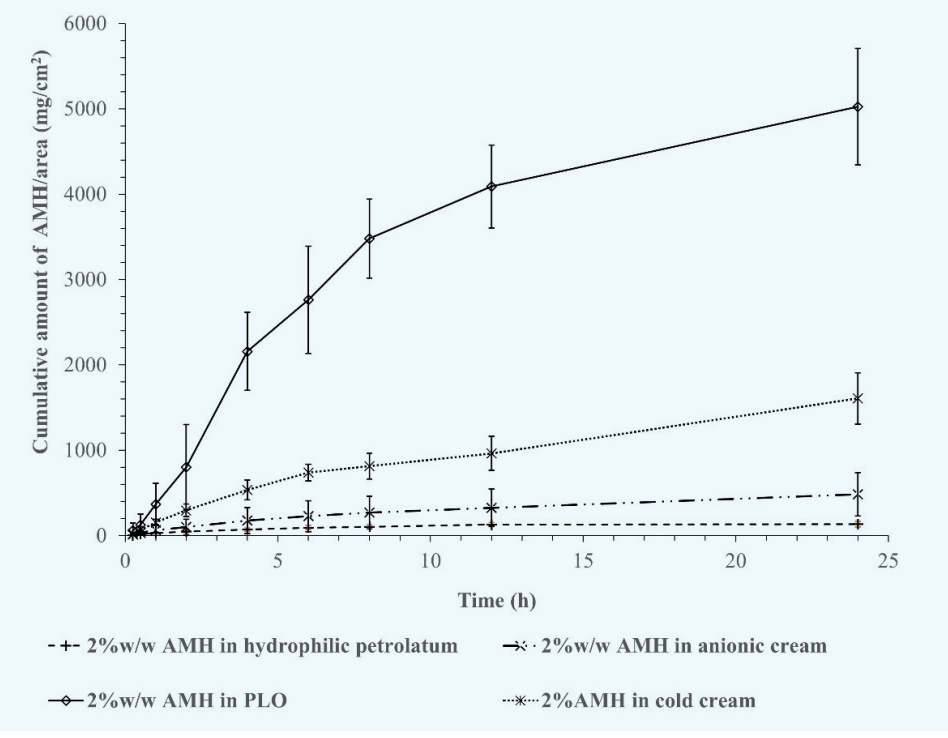

Fig 3. *In vitro* release profiles of AMH from 2%w/w AMH in various compounding bases. Data represent the mean ± SD (n=4-5).

Moreover, the drug molecules had limited mobility due to the viscosity of the ointment base. Consequently, the dissolution and dissociation of AMH likely occurred at or near the physical boundary.[28] Together with the low water solubility of amitriptyline, the result was that the release of amitriptyline from the hydrophilic petrolatum was the lowest.

In vitro skin permeation study

Based on the *in vitro* release studies, it was found that cold cream and PLO are suitable for further skin permeation studies to examine the possibility of using AMH extemporaneous preparations as topical analgesics.

Fig 4 shows the cumulative amount of amitriptyline expressed as AMH in µg/cm^2 transferred from PLO and cold cream to the receptor compartment of citrate buffer pH 5.5. Throughout the experimental period of 24 h, the PLO showed significantly higher levels of amitriptyline skin permeability (37.4 ± 6.9 µg/cm^2) than the cold cream (20.3 ± 5.2 µg/cm^2). These results seem to confirm the conclusions reached by several studies that the PLO provides enhancement of drug transport into or across the skin and is thus widely used in pharmaceutical compounding to enhance the skin permeability of many therapeutic drugs.[26] The permeation enhancement effect of PLO is from its structural matrix. The PLO is a colloidal system

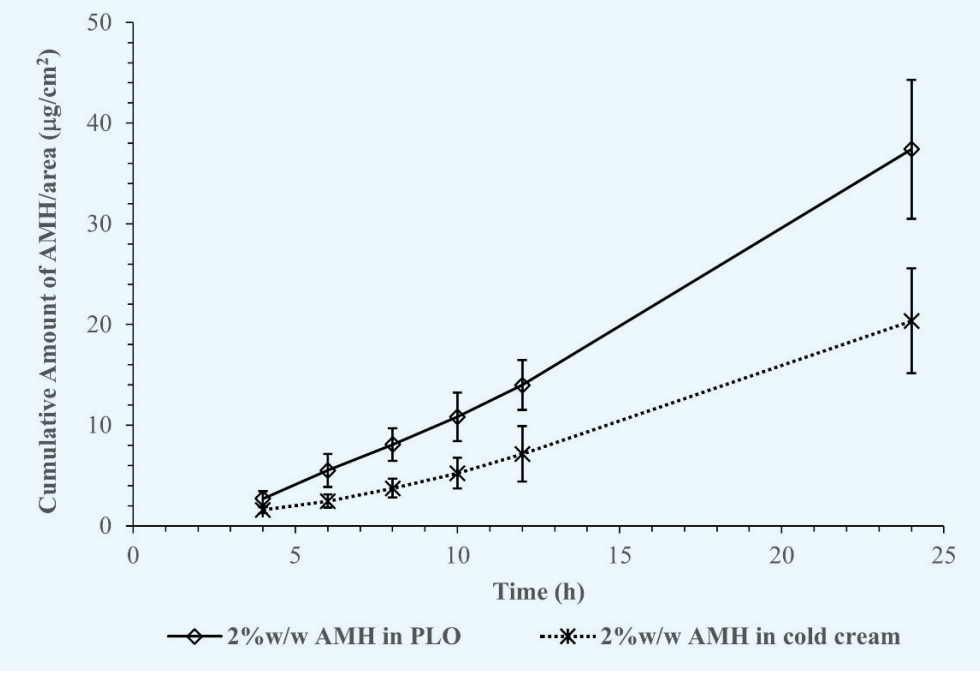

Fig 4. *In vitro* drug permeation profiles of AMH from 2%w/w AMH in PLO and 2%w/w AMH in cold cream. Data represent the mean ± SD (n=5).

that cylindrical inverted micelles of lecithin entrapped in the three-dimensional network of the external aqueous phase.[29] The micelles of the surfactant disorganize the stratum corneum, promoting lipid fluidity, decreasing the barrier function and enhancing drug permeation through the skin.[30]

Stability study

Physical stability testing

The organoleptic properties of AMH compounded in cold cream and PLO remained relatively consistent in all storage conditions. However, AMH compounded in cold cream was slightly more viscous but still spreadable after 60 days stored at 2–8 °C. The same consistency was observed when the cold cream formulations were kept at 30 °C and 40 °C after 14 days. However, regarding the viscosity of the formulations kept at room temperature, AMH in cold cream had a lower viscosity (5,900 cps to 10,900 cps) than the AMH prepared in PLO (124,000 cps to 136,000 cps), as shown in Fig S1A and S1B, respectively. The viscosity of AMH in cold cream was significantly increased when kept at higher temperatures, namely 30 °C for 60 days and 40 °C for 30 days (5,900 cps to 10,900 cps) ($p<0.05$). For the AMH in PLO, the viscosity presented a slight change after being kept at 30°C (124,000 cps to 136,000 cps), and significant increasing after being kept at 40 °C (132,000 cps to 220,000 cps) for 60 days ($p<0.05$).

Chemical stability testing

The percentage of the initial concentration of AMH in the formulations at each time point and storage condition are summarized in Table 2. AMH in cold cream base stored at 2–8 °C and 30 °C, had a significant decrease in the percentage of drug remaining after day 14 compared to day 0 ($p < 0.05$). However, after 60 days, the percentage of drug remaining was still within the acceptable range of 90–110 % of the initial AMH concentration. When AMH in cold cream preparations were stored at 40°C, there was a significant decrease in the percentage of drug remaining after day 14 and 30 ($p < 0.05$). Since the phase separation was detected in AMH in the cold cream after being stored at 40 °C for 60 days, i.e. some oil spreading on the surface of the preparation (Fig S2), the percentage of drug remaining was not reported. However, AMH compounded in PLO showed a significant increase in drug concentration after being stored at both 30 °C and 40 °C compared to day 0 ($p < 0.05$). After 30 days, the percentage of drug remaining of AMH at both 30 °C and 40 °C exceeded the acceptable range of drug remaining. This may be due to water loss from non-airtight plastic containers used in the study, resulting in increased potency of AMH.

Microbiological stability

The microbial content of the formulations of AMH in cold cream and AMH in PLO were determined. According to USP41, Chapter <1111> Microbial Examination of Nonsterile Product Acceptance Criteria for Pharmaceutical Preparations and Substances for Pharmaceutical Use, the total aerobic microbial count must have ≤200 cfu/g after the plate is incubated at 30 °C to 35 °C for 3 days. The total combined yeasts and molds count (must have ≤ 200 cfu/g after the plate is incubated at 20 °C to 25 °C

TABLE 2. Percentage of AMH (% drug remaining) in cold cream and PLO under various storage conditions.

Base	Condition	% Drug remaining			
		Day 0	Day 14	Day 30	Day 60
Cold cream	2-8 °C	100.0 ± 0.0	95.8 ± 4.2	91.0 ± 2.7	95.8 ± 1.3
	30 °C	100.0 ± 0.0	96.0 ± 3.5	93.9 ± 3.9	96.9 ± 3.4
	40 °C	100.0 ± 0.0	97.0 ± 3.4	98.1 ± 2.9	NA
PLO	30 °C	100.0 ± 0.0	109.8 ± 2.9	110.5 ± 2.2	116.4 ± 1.4
	40 °C	100.0 ± 0.0	109.4 ± 1.7	112.6 ± 1.3	119.5 ± 2.6

Data represent mean ± SD (n = 4).
*NA = not available

for 5 days. Both formulations complied with the USP41 standard. After 60 days of the study period, both formulations in all conditions above were examined. Microbial growth was absent in all samples tested.

We were able to prepare 2%w/w AMH in hydrophilic petrolatum USP, anionic cream, cold cream USP and PLO. Significant drug release from the cold cream and PLO was found. Compounded preparations of AMH in cold cream and PLO were further studied for *in vitro* permeation and stability. The compounded preparations of AMH in cold cream were stable at 2–8 °C and 30 °C for 60 days and at 40°C for 30 days, whereas AMH in PLO were stored and found to be stable at 30 °C and 40 °C for 14 days. The organoleptic properties of AMH compounded in cold cream and PLO remained relatively consistent, and the AMH remaining concentration was within the range of 90–110% in all storage conditions. There was no visible microbial growth in any of the sample.

Shakshuki et al.[31] investigated *in vitro* amitriptyline permeation across simulated skin from 1%,5% and 10% formulations in each of 3 bases (Lipoderm base, Emollient cream, and Mediflo 30 PLO). They found that amitriptyline 5% or 10% compounded in Lipoderm base or Emollient cream has the highest drug permeation. Kung et al.[32] determined skin drug delivery across porcine skin from a 4% amitriptyline formulation comprising isopropyl alcohol combined with propylene glycol or isopropyl myristate. They found that the high concentration of isopropyl alcohol in isopropyl myristate/isopropyl alcohol binary formulations greatly contributes to an increased skin permeation of amitriptyline. Based on the data generated through permeation experiments from previous and our studies, the higher drug permeation could potentially result in greater therapeutic efficacy. Available evidence supports the effectiveness of topical amitriptyline alone and in combination with other agents (i.e, ketamine and lidocaine) in the treatment of neuropathic pain.[12,33] Therefore, it is expected that the AMH topical formulations developed in this study could be used in clinical practice.

CONCLUSION

Based on the results of *in vitro* permeation and product stability studies in the present study, it is suggested that AMH in cold cream could be prepared and used as extemporaneous topical preparations with a beyond-use date of 60 days when kept at 2–8 °C and 30 °C. AMH in PLO can also be prepared and used as extemporaneous topical preparations with a beyond-use date of 14 days when kept at 30 °C, which is a shorter stability period than that of AMH in cold cream. Changing the plastic containers to glass containers or airtight containers may increase the beyond-use date, leading to significant cost savings for patients as these products tend to be expensive.

Declaration of conflicts of interest

The authors declare that they have no conflicts of interest with the manufacturers or suppliers of any of the products or materials in this study.

ACKNOWLEDGEMENTS

This research project is supported by Mahidol University (Basic Research Fund: fiscal year 2022) (Grant No. BRF1-030/2565). The authors would like to express our deep appreciation to Associate Professor Dr. Chuthamanee Suthisisang for her suggestion of the idea for this research project.

REFERENCES

1. Loo M. Chronic Pain. In: Loo M, editor. Integrative Medicine for Children. St. Louis, Missouri, USA: Elsevier; 2009. p.238-44.
2. Jackson TP, Stabile VS, McQueen KAK. The global burden of chronic pain. ASA Monitor. 2014;78(6):24-7.
3. Gilron I, Watson CPN, Cahill CM, Moulin DE. Neuropathic pain: a practical guide for the clinician. Can Med Assoc J. 2006;175(3): 265-75.
4. Park TJ, Shin SY, Suh BC, Suh EK, Lee IS, Kim YS, et al. Differential inhibition of catecholamine secretion by amitriptyline through blockage of nicotinic receptors, sodium channels, and calcium channels in bovine adrenal chromaffin cells. Synapse. 1998;29(3):248-56.
5. Eisenach JC, Gebhart GF. Intrathecal amitriptyline acts as an N-methyl-D-aspartate receptor antagonist in the presence of inflammatory hyperalgesia in rats. Anesthesiology. 1995;83(5): 1046-54.
6. Traiffort E, Pollard H, Moreau J, Ruat M, Schwartz JC, Martinez-Mir MI, et al. Pharmacological characterization and autoradiographic localization of histamine H2 receptors in human brain identified with [125I]iodoaminopotentidine. J Neurochem. 1992;59(1): 290-9.
7. Brueckle MS, Thomas ET, Seide SE, Pilz M, Gonzalez-Gonzalez AI, Nguyen TS, et al. Adverse drug reactions associated with amitriptyline — protocol for a systematic multiple-indication review and meta-analysis. Syst Rev. 2020;9(1):59-67.
8. Hansch C, Leo A, Hoekman D. Exploring QSAR: Hydrophobic, electronic, and steric constants In: Hansch C, Leo A, Hoekman D, editors. Exploring QSAR: Hydrophobic, electronic, and steric constants 2. Washington, DC American Chemical Society; 1995.
9. Liebregts R, Kopsky DJ, Hesselink JMK. Topical amitriptyline in post-traumatic neuropathic pain. J Pain Symptom Manage. 2011;41(4):e6-7.
10. Kiani J, Nasrollahi SA, Esna-Ashari F, Fallah P, Sajedid F. Amitriptyline 2% cream vs. capsaicin 0.75% cream in the treatment of painful diabetic neuropathy (double blind, randomized clinical trial of efficacy and safety). Iran J Pharm Res. 2015;14(4):1263–8.
11. Casale R, Symeonidou Z, Bartolo M. Topical treatments for localized neuropathic pain. Curr Pain Headache Rep. 2017;21(3): 15-23.
12. Lynch ME, Clark AJ, Sawynok J, Sullivan MJL. Topical 2% amitriptyline and 1% ketamine in neuropathic pain syndromes: a randomized, double-blind, placebo-controlled trial. Anesthesiology. 2005;103(1):140-6.
13. Thompson DF, Brooks KG. Systematic review of topical amitriptyline for the treatment of neuropathic pain. J Clin Pharm Ther. 2015;40(5):496-503.
14. Chantasart D, Chootanasoontorn S, Suksiriworapong J, Li SK. Investigation of pH influence on skin permeation behavior of weak acids using nonsteroidal anti-inflammatory drugs. J Pharm Sci. 2015;104(10):3459-70.
15. Allen LV. Oinments, creams, and pastes. In: Allen LV, editor. The art, science, and technology of pharmaceutical compounding. 4th ed. Washington, DC, USA: American Pharmacists Association; 2012. p.265-83.
16. Yang Y, Wang S, Xu H, Sun C, Li X, Zheng J. Properties of topically applied organogels: rheology and in vitro drug release. Asian J Pharm Sci. 2008;3(4): 175-83.
17. Mady FM, Essa H, El-Ammawi T, Abdelkader H, Hussein AK. Formulation and clinical evaluation of silymarin pluronic-lecithin organogels for treatment of atopic dermatitis. Drug Des Devel Ther. 2016;10:1101-10.
18. Bourdon F, Lecoeur M, Leconte L, Ultré V, Kouach M, Odou P, et al. Evaluation of Pentravan®, Pentravan® plus, Phytobase®, Lipovan® and pluronic lecithin organogel for the transdermal administration of antiemetic drugs to treat chemotherapy-induced nausea and vomiting at the hospital. Int J Pharm. 2016;515(1-2):774-87.
19. Ibrahim MM, Hafez SA, Mahdy MM. Organogels, hydrogels and bigels as transdermal delivery systems for diltiazem hydrochloride. Asian J Pharm Sci. 2013;8:48-57.
20. International Conference On Harmonisation. Q2(R1) Validation of Analytical Procedures: Text and Methodology. Guidance for Industry. 2005
21. Chantasart D, Tocanitchart P, Wongrakpanich A, Teeranachaideekul V, Junyaprasert VB. Fabrication and evaluation of Eudragit® polymeric films for transdermal delivery of piroxicam. Pharm Dev Technol. 2018;23(8):771-9.
22. United States Pharmacopeial Commission. United States Pharmacopeia and National Formulary, USP41-NF36. Rockville, MD: United States Pharmacopeial Convention, Inc. 2018.
23. Allen LV, Bassani GS, Elder EJ, Parr AF. Strength and stability testing for compounded preparations. Rockville, MD, USA: US Pharmacopeia; 2014. p.1-7.
24. Sri-in J, Sisan W, Kingkhangphloo P, Jutasompakorn P, Chandranipapongse W, Chatsiricharoenkul S, et al. Stability and sterility of extemporaneously prepared 0.01% atropine ophthalmic solution in artificial tears and balanced salt solution. Siriraj Med J. 2022; 74(2):91-9.
25. Carafa M, Santucci E, Lucania G. Lidocaine-loaded non-ionic surfactant vesicles: characterization and in vitro permeation studies. Int J Pharm. 2002;231(1):21-32.
26. Alsaab H, Bonam SP, Bahl D, Chowdhury P, Alexander K, Boddu SH. Organogels in drug delivery: A special emphasis on pluronic lecithin organogels. J Pharm Pharm Sci. 2016;19(2): 252-73.
27. Gupta S. Biocompatible microemulsion systems for drug encapsulation and delivery. Curr Sci. 2011;101(2):174-88.
28. Frenning G. Theoretical investigation of drug release from planar matrix systems: effects of a finite dissolution rate. J Control Release. 2003;92(3):331-9.
29. The history of pluronic lecithin organogel: An interview with Marty Jones, BSPharm, FACA, FIACP. Int J Pharm Compound. 2003;7(3):180-6.
30. Pandey A, Mittal A, Chauhan N, Alam S. Role of surfactants as penetration enhancer in transdermal drug delivery system. J mol Pharm Org Process Res. 2014;2(2):113-22.
31. Shakshuki A, Yeung P, Agu RU. Compounded topical amitriptyline for neuropathic pain: in vitro release from compounding bases and potential correlation with clinical efficacy. Can J Hosp Pharm. 2020;73(2):133-.40.
32. Kung CP, Sil BC, Zhang Y, Hadgraft J, Lane ME, Patel B, et al. Dermal delivery of amitriptyline for topical analgesia. Drug Deliv Transl Res. 2022;12(4):805-15.
33. Uzaraga I, Gerbis B, Holwerda E, Gillis D, Wai E. Topical amitriptyline, ketamine, and lidocaine in neuropathic pain caused by radiation skin reaction: a pilot study. Support Care Cancer. 2012;20(7):1515-24.