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POSSIBILITY ASSESSMENT OF WATER-IN-OIL MICROEMULSION FOR DERMAL DELIVERY OF PIROXICAM

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Abstract: This study aimed to assess the possibility of water-in-oil (w/o) microemulsion (ME) for dermal delivery of piroxicam in terms of solubility power and *in vitro* release. Six blank MEs were prepared from different ratios of Tween 80, Span 80, isopropyl palmitate, isopropyl alcohol and water. A blank ME providing the highest piroxicam solubility was selected for preparation of piroxicam-loaded ME. The obtained product was investigated for tendency of stability when kept in light-protected glass containers at room temperature (28 ± 2°C) for two months. It was then evaluated *in vitro* drug release through dialysis membrane by modified Franz diffusion cells comparing with 0.5% w/w piroxicam commercial gel. The results indicated that piroxicam solubility in MEs was related to solubilization ability of the components. According to solubility data, piroxicam-loaded ME could be prepared with drug content of only 0.3% w/v. It was stable under the investigated conditions. *In vitro* release data indicated that the piroxicam-loaded ME could provide gradual drug release; however, a lag time of 0.5 h was observed and the amount of released drug was significantly lower than that from the commercial gel. In conclusions, the studied w/o ME could be used for production of 0.3% piroxicam-loaded ME having high tendency of stability. Nevertheless, the drug solubility and release results exhibited that this w/o ME was unsuitable for piroxicam incorporation. The interaction between drug and components as well as microstructure of ME may be a crucial parameter for therapeutic efficacy since it could adversely influence drug solubility and release.

Keywords: microemulsion, NSAIDs, piroxicam, solubility, drug release

INTRODUCTION

Piroxicam is one of non-steroidal anti-inflammatory drugs (NSAIDs) being used for the treatment of acute and chronic rheumatoid arthritis. Although it possesses high therapeutic efficacy, many systemic side effects including gastrointestinal irritation, dizziness, headache and peptic ulcer have been found following oral administration (Lipscomb, Wallis, Armstrong, & Rees, 1998; National Center for Biotechnology Information, 2018). Since 2007, the European Medicines Agency has recommended restrictions on the use of piroxicam-containing medicinal products; however, topical medicines incorporating this drug are not concerned by these restrictions (European Medicines Agency, 2007). Therefore, development in an effective formulation for dermal delivery of piroxicam is challenging. Although piroxicam gels have been commercially available in many trade names, liquid dosage form of this drug is interesting to be alternatively used as topical spray.

Microemulsion (ME) is an interesting dermal drug delivery system since it can provide potential for skin penetration enhancement, spontaneous formation, thermodynamic stability

and good appearance (Lopes, 2014; Souto, Doktorovova, & Boonme, 2011). Therefore, it is widely used as a topical vehicle for several drugs, including many NSAIDs (Singh, Sharma, Veerma, Javed, & Singh, 2013). Some formulations of piroxicam-loaded oil-in-water (o/w) MEs and microemulsion-based gels (MBGs) have been previously investigated and reported for topical administration. An optimum o/w ME containing 0.5% piroxicam, 10% Labrasol, 50% ethanol, 10% oleic acid and water to 100% was found to provide the permeation rate of 47.14 μg/cm^2/h through Sprague-Dawley rat skin (Park, Cui, Yun, Ko, & Chi, 2005). An o/w ME consisted of 26.5% Tween 80, 55.0% ethanol, 7.5% cas tor oil and 11.0% phosphate buffer was reported that it could dissolve 1.0% piroxicam due to the help of lipophilic component of ME and lipophilic part of surfactant (Nazar, Khan, & Shah, 2009). ME composed of 40% Tween 80, 20% propylene glycol, 6.67% oleic acid and 33.33% water could be incorporated with 0.5% piroxicam and exhibited desirable characteristics in terms of particle size and stability (Abd-Allah, Dawaba, & Ahmed, 2010a). Hydrophilic gel bases (i.e., methyl cellulose, carboxy methyl cellulose, hydroxypropyl methyl cellulose, Carbopol 934, Carbopol 940 and Pluronic F-127) containing piroxicam-loaded ME were compared for drug release with those containing piroxicam plain drug. It was reported that piroxicam in form of ME could enhance drug release (Abd-Allah, Dawaba, & Ahmed, 2010b). Although previous investigations about piroxicam-loaded MEs were focused on only o/w type, it was proved that water-in-oil (w/o) MEs could increase the percutaneous absorption of diclofenac sodium, a NSAID (Songkro et al., 2012). Thus, it was interesting to formulate new w/o ME for piroxicam incorporation. The objective of this study was to assess the possibility of w/o ME for dermal delivery of piroxicam in terms of solubility power and *in vitro* release.

MATERIALS AND METHODS

Materials

Piroxicam, Tween 80 (polyoxyethylene 20 sorbitan monooleate) and Span 80 (sorbitan monooleate) were purchased from PC Drug Center (Bangkok, Thailand). Isopropyl palmitate (IPP) was obtained from East Asiatic (Bangkok, Thailand). Isopropyl alcohol (IPA), methanol, hydrochloric acid (HCl) and sodium chloride were procured from RCI Labscan Asia (Bangkok, Thailand). Potassium dihydrogen phosphate, disodium hydrogen phosphate and potassium chloride were purchased from Univar (New South Wales, Australia). All chemicals were pharmaceutical grade and used as received without any modifications. The 0.1 M methanolic HCl, isotonic phosphate buffer solution pH 7.4 (PBS) and distilled water were prepared in-house. A 0.5% w/w piroxicam commercial gel was bought from a drug store in Songkhla Province, Thailand.

Preparation and type determination of blank MEs

The ME region in the pseudoternary phase diagram of nonionic system composed of 1:1 mixture of Tween 80 and Span 80 as surfactant blend, IPP as oil phase and 1:2 mixture of IPA and water as aqueous phase was used for preparation of blank MEs (Boonme, Boonthongchuay, Amnuaikit, & Wongpoowarak, 2013). Six points in this ME region were selected to prepare six blank MEs as exhibited in Table 1 by simply mixing. These formulations were composed of either 40% or 50% surfactant blend and the weight ratios between oil phase and aqueous phase were varied as 1:1, 3:1 and 1:3. They were observed for visual appearance. Their type was determined by drop dilution test. Briefly, each sample was dropped into Ponceau 4R aqueous solution. Immiscibility was expected to be observed if the sample was w/o ME.

Solubility study of piroxicam

Excess amount of piroxicam powder was added into 5 mL of each blank ME and then constantly shaken in the shaking bath which the temperature was controlled at 25 ± 1°C for 72 h. Afterwards, the obtained suspension was filtered. The filtrate was analyzed for amount of solubilized piroxicam after the appropriate dilution with 0.1 M methanolic HCl. The solubility of piroxicam in pure Tween 80, Span 80, IPP, IPA and water was also evaluated in the identical manner as aforementioned. The experiments were performed in triplicate.

Preparation and stability study of piroxicam-loaded ME

A blank ME which provided the highest solubility of piroxicam was selected for incorporation with the drug in a suitable concentration according to the obtained solubility results. The prepared piroxicam-loaded ME was observed for visual appearance. Its type was determined by drop dilution test. Its globular structure was confirmed by observation under transmission electron microscope (TEM; JEM-2010, JEOL, Japan). Briefly, piroxicam-loaded ME was dropped on a Formvar carbon film on 200-mesh copper grid and left at room temperature (28 ± 2°C) until dry. Finally, the obtained sample was observed under TEM at the magnification of ×100,000. Droplet size was also determined from the obtained TEM photograph by an image analyzer. Drug content in piroxicam-loaded ME was analyzed after preparation and after kept in glass containers with light protection at room temperature for two months. Briefly, piroxicam was extracted from ME by mixing with 0.1 M methanolic HCl and then filtered. The obtained filtrate was diluted into appropriate concentration with 0.1 M methanolic HCl and then analyzed. The experiments were carried out in triplicate.

In vitro release study of piroxicam-loaded ME

In vitro drug release of piroxicam-loaded ME was investigated by modified Franz diffusion cells (Hanson Model 57-6 M, Hanson Research Corporation, Los Angeles, CA) and compared with that of a piroxicam commercial gel. Dialysis membrane (Cellu Sep®, Membrane filtration Products, Inc., Seguin, TX) was used as the membrane model. It was cut into appropriate flat piece and boiled in water for 20 min. After cool down to room temperature, the membrane was soaked in PBS for 8 h before used. The hydrated membrane was mounted between donor and receptor compartments of each diffusion cell with an effective release area of 1.77 cm^2. The receptor compartment was filled with 12 mL of degassed PBS and continuously stirred at a speed of 200 rpm by a magnetic bar. The diffusion cells were connected to a circulating water bath thermostated at 37 ± 0.5°C. After the membrane was equilibrated for 30 min, 1 mL of piroxicam-loaded ME or 1 g of piroxicam commercial gel was placed onto the membrane in the donor compartment. At defined time intervals (0, 0.5, 1, 2, 3, 4 and 5 h), 1 mL of receptor fluid was taken from each receptor compartment and immediately replaced with an equal volume of fresh PBS. The withdrawn samples were analyzed to find cumulative amount of released piroxicam. The experiments were done in triplicate.

Analytical method of piroxicam

The drug amounts from the parts of solubility, stability and release studies were analyzed by UV spectrophotometry (Dhage, Chhabra, & Banerjee, 2011). Briefly, the samples were measured for the absorbance values at the wavelength of 334 nm in triplicate by UV spectrophotometer (Spectronic Genesys 5, Milton Roy, Ivyland, PA) and 0.1 M methanolic HCl was used as the blank for zero setting. Finally, the drug amounts were calculated comparing with the calibration curve. In validation study, the calibration curve between absorbance values versus concentrations of piroxicam standard solutions at 3, 4, 5, 6 and 7

μg/mL was found to follow Beer-Lambert's law, with regression (r^2), slope and intercept of 0.999, 0.075 and 0.016, respectively.

RESULTS AND DISCUSSION

Characteristics of blank MEs

All prepared blank MEs (ME1-ME6) could be easily obtained by simply mixing due to spontaneous formation, a specific property of MEs. Their visual appearance was clear yellowish liquid. They were immiscible with Ponceau 4R aqueous solution, indicating for w/o type. Total hydrophilic-lipophilic balance (HLB) value of the surfactant blend in these six formulations was 9.65 which was lower than 10, resulting in negative curvature of the interfacial film and formation of w/o MEs (Mehta & Kaur, 2011). Nicotinamide-loaded ME, which was composed of 3% w/w nicotinamide, 50% w/w 1:1 mixture of Tween 80 and Span 80, 20% w/w IPP and 27% w/w 1:2 mixture of IPA and water, has been previously reported as w/o ME without birefringence when observed under polarized light microscope (Boonme, Boonthongchuay, Wongpoowarak, & Amnuaikit, 2016).

Solubility of piroxicam

Solubility of piroxicam in among six blank MEs was in the range of ME5 > ME4 > ME1 > ME2 > ME3 > ME6 as shown in Table 1. The reason was unclear; however, it seemed to be related to combination effects on piroxicam solubility of different ME components. Piroxicam could be solubilized in pure Tween 80, Span 80, IPP, IPA and water as 0.590 ± 0.001%, 0.113 ± 0.001%, 0.076 ± 0.000%, 0.298 ± 0.005% and 0.002 ± 0.000% w/v, respectively. In previous reports, the components in MEs were found to influence on the solubilization of the loaded drugs (Boonme, Wuttikul, Fookloy, Promjan, & Teeranachaideekul, 2017; Nandi, Bari, & Joshi, 2003). Additionally, when comparing at the same 50% surfactant blend, piroxicam solubility ability of MEs increased when amounts of oil phase increased (ME4 > ME2 > ME6). In contrast, when comparing at the same 40% surfactant blend, piroxicam solubility ability of MEs increased when amounts of oil phase decreased (ME5 > ME1 > ME3). Hence, the current results implied that solubility of piroxicam, a lipophilic drug, in the studied MEs may be also associated with surfactant aggregation and oil penetration at interfacial film.

Solubility of piroxicam in all MEs was lower than that in Tween 80. Furthermore, solubility of piroxicam in blank MEs, except ME5, was less than that in IPA. Although MEs are generally known for high solubilization ability, a competitive situation between oil phase and lipophilic drug for the same solubilization site (lipophilic tails of surfactant film) may lead to a decrease in overall drug solubility in MEs. For example, it was reported that small molecular volume oils could penetrate the lipophilic chains of the interfacial surfactant film of MEs. Thus, presence of small molecular volume oils in MEs could not enhance the incorporation capacity for testosterone propionate (a lipophilic drug) although they presented high solubilization for this drug (Malcolmson, Satra, Kantaria, Sidhu, & Lawrence, 1998). Moreover, piroxicam was confirmed to be localized in the interfacial film of ME composed of Tween 80, ethanol, castor oil and phosphate buffer when studied with fluorescence spectrum (Nazar et al., 2009).

Table 1. Formulations of blank MEs and piroxicam solubility in MEs.

Formulation	Component (% w/w)			Piroxicam solubility (% w/v)
	1:1 Tween 80:Span 80	IPP	1:2 IPA:water	
ME1	40.0	30.0	30.0	0.230 ± 0.001
ME2	50.0	25.0	25.0	0.216 ± 0.001
ME3	40.0	45.0	15.0	0.206 ± 0.001
ME4	50.0	37.5	12.5	0.284 ± 0.001
ME5	40.0	15.0	45.0	0.360 ± 0.001
ME6	50.0	12.5	37.5	0.190 ± 0.001

All studied MEs provided piroxicam solubility much lower than 0.5% which has been the drug content of generally marketed gels. However, an effective amount of piroxicam in topical anti-inflammatory products has been acceptable in range of 0.3% to 2.0% (Nozawa, Ohaya, & Kawazoe, 1987; Shin & Bossuyt, 1995). Therefore, ME5 was selected to further prepare 0.3% w/v piroxicam-loaded ME.

Characteristics and stability of piroxicam-loaded ME

The obtained piroxicam-loaded ME was clear yellowish liquid which was similar to its blank counterpart as shown in Figure 1(a). Additionally, it was confirmed for w/o type since immiscibility was observed after it was dropped into Ponceau 4R aqueous solution as illustrated in Figure 1(b). Nano-sized globules were observed under TEM as exhibited in Figure 1(c). After piroxicam-loaded ME was kept in light-protected glass containers for two months, no changes in physical appearance such as phase separation, turbidity and precipitate formation were found. Its drug content was 98.30 ± 0.02% of the initial concentration, implying to possibly high tendency of stability.

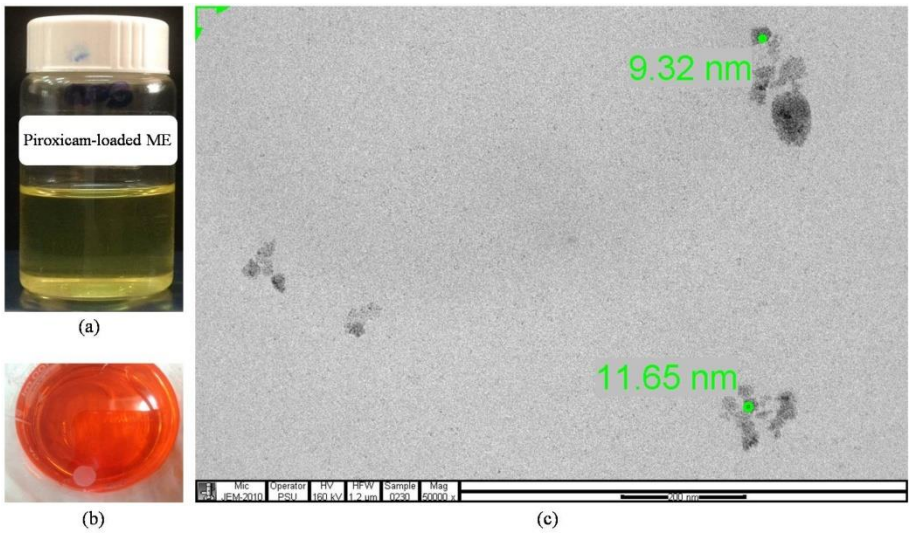


Figure 1. Piroxicam-loaded ME: (a) visual appearance, (b) immiscibility result after drop dilution test into Ponceau 4R aqueous solution and (c) TEM photograph at magnification of ×100,000.

In vitro release of piroxicam-loaded ME

It could be seen in Figure 2 that the investigated ME provided undetectable amounts of released piroxicam at the initial lag time of 0.5 h and subsequently slow release profile. Amount of released piroxicam from the prepared piroxicam-loaded ME was detected to be 28.34 ± 2.02% of initial concentration per cm^2 of effective release area at the end of *in vitro* release study. In contrast, piroxicam commercial gel provided no lag time and amount of released drug was 31.11 ± 0.32% of theoretical concentration per cm^2 of effective release area at the end of *in vitro* release study. PBS was used as a receptor fluid; therefore, low solubility of piroxicam in water, solvent of PBS, resulted in low amounts of released drug from both ME and gel. Nevertheless, under the same condition, piroxicam-loaded ME provided significantly lower amounts of released drug than piroxicam commercial gel at all determined time intervals ($p < 0.05$, t-test). Higher variation was also found in the release profile of piroxicam-loaded ME.

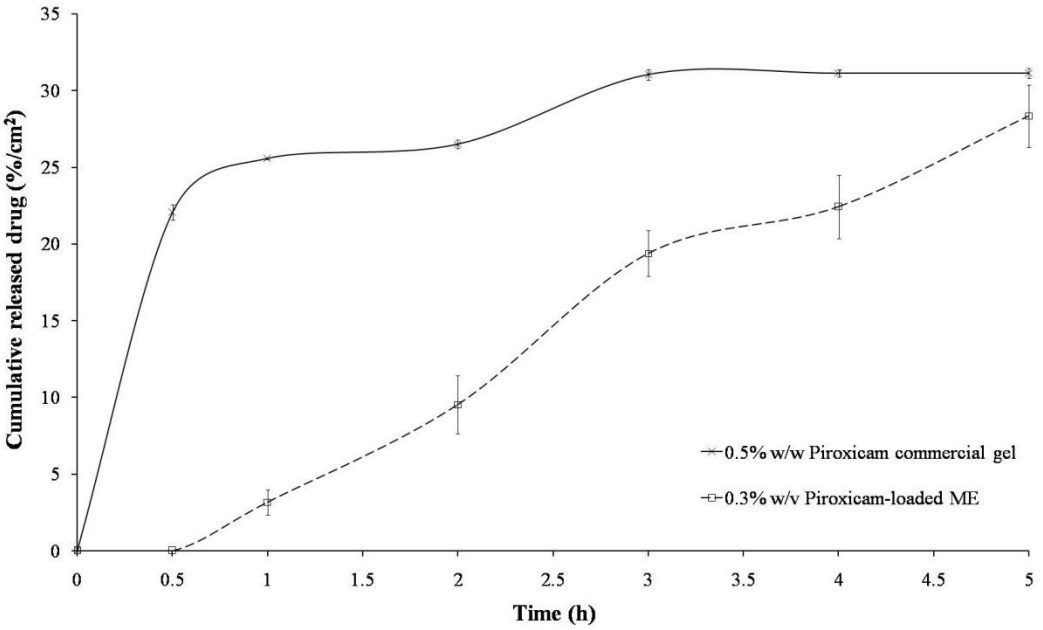


Figure 2. *In vitro* release profiles of 0.3% w/v piroxicam-loaded ME and 0.5% w/w piroxicam commercial gel.

Although ME systems are generally known for many advantages in dermal drug delivery, the interaction between drug and ME components as well as ME microstructure has to be concerned since it led to low drug release. For instance, strong surfactant/cosurfactant synergism was found as the cause of slow release of ibuprofen incorporated in ME systems composed of Labrasol as surfactant, either Solubilisant gamma 2421 or Solubilisant gamma 2429 as cosurfactant, isopropyl myristate as oil phase and water as aqueous phase (Djekic, Primorac, Filipic, & Agbaba, 2012). It was previously reported that piroxicam was localized in the palisade layer of the interfacial film of ME formulated using Tween 80 as surfactant; however, release characteristics were not investigated yet (Nazar et al., 2009). Lipophilic piroxicam in the current ME may locate in lipophilic tails of Tween 80 and Span 80 with high affinity, resulting in difficulty of drug release into hydrophilic PBS. Therefore, lag time and low amount of released drug were found in release profile of piroxicam-loaded ME. In contrast, lag time was not found in the release profile of the commercial gel due to weak affinity between the drug and ingredients in the gel formulation. Lag time was previously not

observed in the *in vitro* cellulose acetate membrane transport profiles of piroxicam from two o/w nanocreams and a commercial gel (Abdulkarim et al. 2010).

CONCLUSION

Among six studied MEs, ME5 containing 40% w/w 1:1 mixture of Tween 80 and Span 80 as nonionic surfactant blend, 15% w/w IPP as oil phase and 45% w/w 1:2 mixture of IPA and water as aqueous phase provided the highest piroxicam solubility as 0.360 ± 0.001% w/v. The ability of the studied MEs for incorporation with piroxicam was limited according to intrinsic solubilization ability of the components and competition at the solubilization site. The obtained 0.3% w/v piroxicam-loaded ME was clear yellowish liquid and w/o type like its blank counterpart. It was stable when kept in light-protected glass containers at room temperature for two months. It exhibited significantly slower and less release of piroxicam than the commercial gel. This phenomenon may be caused by high interaction and affinity between the drug and the ME components as well as ME microstructure. Therefore, drug solubility and *in vitro* release should be accessed for screening suitability of ME formulations before further evaluation of therapeutic efficacy.

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