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Enhancement of the Skin Permeation of Clindamycin Phosphate by Aerosol OT/1-Butanol Microemulsions

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Microemulsions of water/isopropyl palmitate (IPP)/Aerosol OT (AOT)/1-butanol were developed as alternative formulations for topical delivery of clindamycin phosphate. Effect of AOT:1-butanol ratios on microemulsion region existence in the pseudo-ternary phase diagrams was investigated. The 2:1 AOT:1-butanol provided the largest microemulsion region. Five microemulsions of 1% w/w clindamycin phosphate were prepared and characterized. The permeation through human epidermis of the microemulsions was evaluated and compared with the 70% isopropanol solution using modified Franz diffusion cells. The drug permeation from all microemulsions was found to be significantly greater than that from the solution, indicating the enhancement of the skin permeation by the microemulsions. Within the same microemulsion type, the drug permeation increased with increasing the amount of AOT:1-butanol. The drug permeation from oil-in-water (o/w) microemulsions was relatively higher than that from water-in-oil (w/o) microemulsions. In addition, all microemulsions were stable for at least three months at 30 ± 1°C.

Keywords microemulsion; clindamycin phosphate; topical delivery; permeation enhancement; inflammatory acne

INTRODUCTION

Microemulsions are thermodynamically stable, transparent, low-viscosity dispersions of oil and water stabilized by an interfacial film of amphiphiles which are usually used surfactants and combined with a cosurfactant such as a medium-chain alcohol. They can be used to deliver drugs via several routes including topical route (Bhargava et al., 1987; Eccleston, 1988; Lawrence & Rees, 2000). Many advantages of microemulsions on topical delivery are generally known, including possibilities of incorporating large amount of drug due to the high solubility power, favoring drug partition into skin by modification of the thermodynamic activity of the drug and reducing diffusion barrier stratum corneum (Delgado-Charro et al., 1997). Moreover, the products in microemulsion dosage form are aesthetic with optical transparence and thermodynamic stability. During the recent decades, microemulsions have been developed and used as topical vehicles for many drugs and cosmetic substances (Baroli et al., 2000; Bolzinger et al., 1998; Changez & Varshney, 2000; Garcia-Celma et al., 1994; Kreilgaard et al., 2000; Linn et al., 1990; Malcolmson & Lawrence, 1993; Peltola et al., 2003; Schmalfuβ et al., 1997; Rhee et al., 2001).

Acne is very common ailment which most frequently develops during the teenage years. When choosing suitable therapy for mild to moderate inflammatory acne, the topical use of antibiotic is a preferred method of treatment. One of antibiotics commonly used for topical treatment of acne is clindamycin phosphate because of its ability to inhibit the growth of *Propionibacterium acnes*, the major factor in the genesis of acne papules and pustules (Anne, 1982; Arnold, 1991; Becker et al., 1981). However, clindamycin phosphate used in topical is commercially available in 50% isopropanol solution (Physicians' Desk Reference, 2003; USP DI, 1998), which may cause skin irritation due to high concentration of alcohol. Since many reports have found that microemulsions could increase skin permeation of drugs as compared to conventional dosage forms or simple solutions (Baroli et al., 2000; Bolzinger et al., 1998; Changez & Varshney, 2000; Kreilgaard et al., 2000; Linn et al., 1990; Peltola et al., 2003), it is of our interest to investigate the permeation enhancement of clindamycin phosphate by using microemulsions as a drug carrier.

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Aerosol OT (AOT) or bis(2-ethylhexyl)sulfosuccinate sodium has been used to increase the intestinal drug absorption of many drugs (Engel & Riggi, 1969; Khalafallah et al., 1975). Attempts have been made to develop AOT microemulsion for oral drug delivery (El-Laithy, 2003). Many studies have extensively used AOT microemulsions for topical drug delivery (Liu et al., 2006; Osborn et al., 1988; Osborn et al., 1991; Trotta et al., 1989) while one reported that AOT microemulsion acts as a safe transdermal carrier (Changez & Varshney, 2000). Therefore, AOT was selected in this study to develop microemulsion of water and isopropyl palmitate (IPP) by using 1-butanol as a cosurfactant to increase the flexibility of the surfactant film around the microemulsion droplet (Alany et al., 2000; Alany et al., 2001). The influence of the microemulsion types, oil-in-water (o/w) or water-in-oil (w/o), on their permeation enhancement of clindamycin phosphate was investigated and compared with the simple solution.

MATERIALS AND METHODS

Materials

Clindamycin phosphate was received as a gift from Genzyme Pharmaceutical (London, UK). AOT was obtained from Fluka (Buche, Switzerland). Water used was sterile water for injection purchased from Thainakornpattana (Bangkok, Thailand). IPP was supplied by Henkel (Düsseldorf, Germany). Potassium dihydrogen phosphate, sodium hydroxide, phosphoric acid, isopropanol and 1-butanol were obtained from Clarlo Erba (Milan, Italy). Methyl paraben, propylene glycol and mineral oil were purchased locally from S. Tong Chemicals (Bangkok, Thailand). Methanol and acetonitrile were obtained from J.T. Baker (New Jersey). All the chemicals were used as received.

Methods

Construction of Phase Diagrams of Microemulsions

The microemulsion systems of water, IPP, AOT and 1-butanol were prepared at different concentration of each ingredient. The phase diagrams were constructed to obtain their concentration ranges that could result in existence area of microemulsion without the drug. The mixtures of AOT and 1-butanol were prepared at various weight ratios; i.e., 1:1, 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, and 9:1. IPP was added to each mixture to obtain (AOT + 1-butanol):IPP weight ratios of 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9. After mixing, the mixture was titrated with an aliquot of water. When transparent liquid formulations were originated, points in the pseudoternary phase diagram were marked. A cut-and-weigh method was used to determine the percentage of the total area of the phase diagram covered by the microemulsions. The system that gave the largest microemulsion region was characterized for their type (o/w or w/o) by the conductivity test using conductivity meter (model 33, Yellow Springs).

Preparation and Characterization of Clindamycin Phosphate Microemulsions

The system of 2:1 AOT:1-butanol weight ratio, which gave the largest microemulsion region, was chosen to prepare clindamycin phosphate microemulsions. Since the drug was added to the system, the weight ratio of AOT and 1-butanol was adjusted to obtain five microemulsion formulations having composition as shown in Table 1. The preparation was performed by incorporating clindamycin phosphate in the internal phase (IPP for o/w type or water for w/o type), adding to the mixture of AOT and 1-butanol, and mixing with the external phase (water for o/w type or IPP for w/o type). All components were mixed with a vortex mixer until the uniform mixture was obtained. Clindamycin phosphate microemulsions were characterized for their type by the dye solubility test with a water-soluble dye (tartrazine) and an oil-soluble dye (dye red), the dilution test with water and mineral oil, and the conductivity test using conductivity meter (model 33, Yellow Springs).

The size of the dispersed phase of the microemulsions was determined by Malvern photon correlation spectrometer model 4700 equipped with an argon laser model 2000 (Malvern Instruments, UK). Light scattering was monitored at an angle of 90° at 30°C, using polystyrene beads to check the instrument's performance. ME1, ME2, and ME3 were o/w type while ME4 and ME 5 were w/o type, therefore the solvent

TABLE 1
Composition (% w/w) and Expected Type of the Studied Microemulsions

Microemulsion						
Formulation	Type	AOT	1-Butanol	IPP	Water	Clindamycin Phosphate
ME1	o/w	15	10	10	64	1
ME2	o/w	30	13	20	36	1
ME3	o/w	33	15	25	26	1
ME4	w/o	15	10	64	10	1
ME5	w/o	30	13	36	20	1

refractive indices and viscosity values used as parameters were those of distilled water and IPP, respectively. The refractive indices of water and IPP were 1.3336 and 1.4378, respectively.

Clindamycin Phosphate Analysis

Clindamycin phosphate was assayed by high pressure liquid chromatography (HPLC) method using a HPLC apparatus consisted of an auto injector (model SIL-10A, Shimadzu, Japan), a pump (model LC-10AD, Shimadzu, Japan) and a UV-Visible detector (model SPD-10AV, Shimadzu, Japan). Methyl paraben (4.8 μg/mL, 20% methanol) was used as an internal standard. The mobile phase, consisting of 0.1 M monobasic potassium phosphate buffer pH 4.0 and acetonitrile (82:18 v/v), was adjusted to flow at a rate of 1.2 mL/min through a reversed phase column C18 (5 μm, 250 × 4.6 mm, Phenomenex). The samples were detected at 214 nm and integrated with RF 10A version 1.1 software LC program. The calibration curves (plots of peak area ratio of drug and internal standard versus drug concentration) were constructed by running standard solutions of the drug and internal standard in phosphate buffer pH 5 for every series of chromatrographed samples. Validation of the method was performed to ensure that the calibration curve between 8 and 48 μg/mL of clindamycin phosphate was in the linearity range and the coefficients of variation were less than 5%, both intra-day and inter-day.

In Vitro Skin Permeation Studies

Permeation studies were carried out on human breast skin using modified Franz cell (Crown Bioscientific, Clinton, NJ) of 1.77 cm^2 surface area and 13.67 mL of the receptor volume. Isolated human epidermis was separated by heat separation technique (Haigh & Smith, 1994). Excess subcutaneous fat and connective tissue were removed from the skin, and the skin was immersed in water at 60°C for 90 sec. Afterwards, the epidermis sheet was carefully separated from the dermis sheet. The isolated human epidermis was kept at −20°C until used. Prior to experiments, the epidermis sheet was hydrated in phosphate buffer pH 5 for 30 min.

The epidermis was then mounted on the receptor compartment with the stratum corneum facing upwards. The other side of the epidermis sheet was contacted with phosphate buffer pH 5 controlled at 37°C in receptor compartment. An amount of 1.5 mL of each microemulsion or the solution was placed onto the epidermis in the donor compartment. At predetermined time (at 1, 2, 3, 5, and 8 hr), 1.5 mL of receptor fluid was taken and analyzed for the amount of permeated clindamycin phosphate by HPLC. An equal volume of fresh receptor fluid was replenished. The experiment was performed in ten replicates (n = 10) for each microemulsion formulation. The drug permeation from clindamycin phosphate microemulsions was compared with that from 1% w/w clindamycin phosphate solution containing 70% w/w isopropanol, 10% w/w propylene glycol and water (Orr et al., 1978).

Statistics

Statistical data were analyzed by one-way analysis of variance (ANOVA). Turkey's multiple comparison test was used to compare different formulations and a P value of 0.05 was considered to be significant.

Stability of Clindamycin Phosphate Microemulsions

The clindamycin phosphate microemulsions were kept for 3 months at 30 ± 1°C. They were then evaluated monthly for the possibility of a change in appearance, pH using pH meter (model SA 520, Orion Research) and phase separation under centrifugal force (6000 rpm, 20 min) using centrifuge machine (model 30F, Hettich Universal Dupont, Germany). The percent remaining of the drug was analyzed at each time interval by HPLC as previously described.

RESULTS AND DISCUSSION

Phase Diagrams

Figure 1 shows the microemulsion region in the pseudoternary phase diagrams of water and IPP at different weight ratios of AOT and 1-butanol. The percentages of the total area of the phase diagram covered by the microemulsions (shaded areas in the peudoternary phase diagram) indicate that the microemulsion region increased when the amount of AOT increased from 1:1 to 2:1 AOT:1-butanol and then decreased when the amount of AOT further increased (from 2:1 to 9:1 AOT:1-butanol). The largest microemulsion region was therefore obtained at 2:1 AOT:1-butanol. These results can be explained that as the amount of surfactant in the system is increased, a greater interfacial area is possible and the oil is distributed among a greater number of micelles. However, if the surfactant and cosurfactant ratio is further increased, clear micellar formulations fail to form because there is an insufficient quantity of the cosurfactant in the system to enable an interfacial film of required composition to be formed (Attwood & Florence, 1983).

The areas of o/w and w/o microemulsion types of the system of 2:1 AOT:1-butanol are shown in Figure 2. It is evident that the w/o type turned to be the o/w type as the percentage of water increased. In opposite, the o/w type turned to be the w/o type as the percentage of IPP increased. Therefore, the results suggest that the microemulsion type of this system depended on weight ratio of water and oil phases.

Characterization of Clindamycin Phosphate Microemulsions

The clindamycin phosphate microemulsions were determined for their type and particle size as reported in Table 2. As expected, the results show that ME1, ME2, and ME3 had miscibility with the water-soluble dye, ability to dilute with water and high conductivity, indicating characteristics of the o/w type. In contrast, ME4 and ME5 possessed opposite properties which were determined to be the w/o type. In addition, the particle size

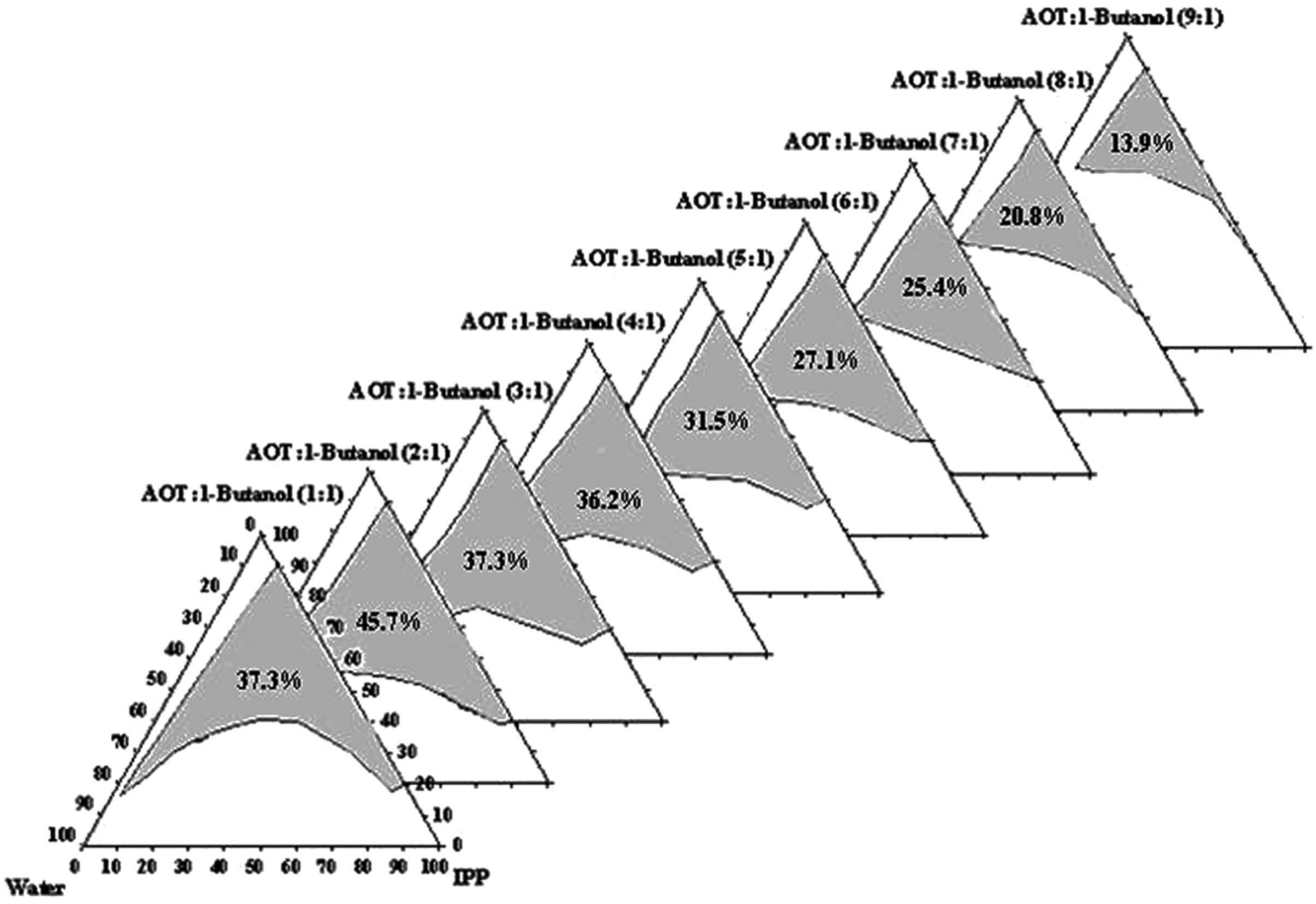

FIGURE 1. Pseudoternary phase diagram of the system of water, IPP at different ratios of AOT and 1-butanol. The numbers shown in the shaded areas represent the percentage of the total area of the microemulsion regions in the phase diagrams.

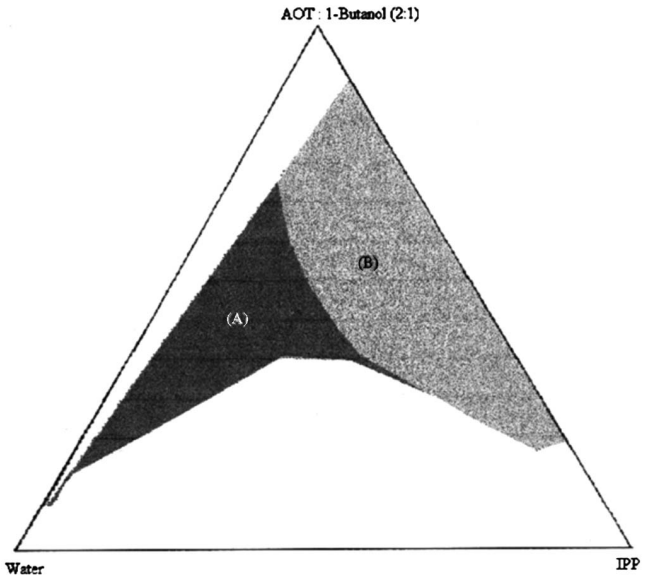

FIGURE 2. Pseudoternary phase diagram of the system of water, IPP and 2:1 AOT:1-butanol. The shaded area (A) represents the o/w microemulsion type, and (B) represents the w/o microemulsion type.

for all microemulsion formulation revealed from the light scattering experiments was in the range of 7–44 nm, which is generally considered to be the particle size of microemulsions (≈10–100 nm) (Bhargava et al., 1987). From Table 2, clindamycin phosphate microemulsions slightly increased the particle size of the internal droplet as compared to those without drug, indicating that the drug would dissolve in the water droplet of the w/o microemulsions or immerse in the surfactant film around the oily droplet of the o/w microemulsions. Of all o/w microemulsions, it was found that the particle size of ME3 was the smallest as compared to those of ME2 and ME1. For w/o microemulsions, the particle size of ME5 was smaller than that of ME4. The results show that the smaller particle size of microemulsions was obtained due to the higher concentration of surfactant and cosurfactant system. The decrease in the particle size can be attributed to the solubilization of internal phase within a larger number of surfactant micelles, which are consequently swollen to a lesser extent (Kale & Allen, 1989).

Skin Permeation Enhancement of Microemulsions

Figure 3 shows the cumulative amount of clindamycin phosphate permeated from ME1 - ME5 and from solution

TABLE 2
Results for the Determination of Microemulsion Type and Particle Size

Formulation	Dye Solubility Test[a]	Dilution Test		Conductivity (μS/cm)	Particle Size (nm)	
		Water Dilution	Oil Dilution		Without Drug[b]	With Drug[b]
ME1	A	Miscible	Immiscible	810	9.4	10.1
ME2	A	Miscible	Immiscible	660	8.6	9.4
ME3	A	Miscible	Immiscible	620	7.3	8.4
ME4	B	Immiscible	Miscible	40	38.4	43.6
ME5	B	Immiscible	Miscible	45	8.8	9.7

[a]A: Miscible with a water-soluble dye; B: Miscible with an oil-soluble dye.
[b]1% w/w Clindamycin phosphate.

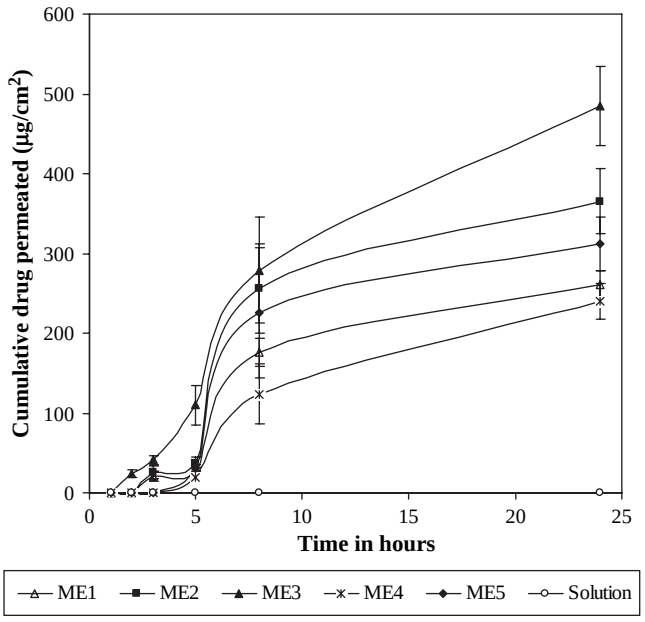

FIGURE 3. In vitro permeation of clindamycin phosphate from microemulsions and the solution (mean ± *SD*, *n* = 10).

through the human epidermis. It should be noted that no permeation of clindamycin phosphate from the solution was observed during 24 hr. Since clindamycin phosphate is freely soluble in water (*British Pharmacopoeia*, 2004), it could dissolve and ionize in the 1% w/w solution. In such case, an ionized clindamycin phosphate would probably not be able to partition through the epidermis due to the lipophilic nature of the stratum corneum. However, the permeated clindamycin phosphate obtain from all microemulsions was drastically higher than that from the solution ($P < 0.05$). Therefore, the microemulsions had enhancement effect on the permeation of clindamycin phosphate through the epidermis. An increase in the drug permeation may be due to the fact that the thermodynamic activity of the drug in the microemulsion can be modified to favor partitioning into the stratum corneum (Delgado-Charro et al., 1997). Additionally, AOT in the microemulsions may act as a permeation enhancer to reduce the diffusional barrier of the stratum corneum.

In comparison of the permeation of all microemulsions for 24 hr, an average efficiency in promoting the percutaneous permeation of clindamycin phosphate tended to be in the following order: ME3 > ME2 > ME5 > ME1 > ME4; however, ME1 and ME4 were not significantly different ($P > 0.05$). From the results, the efficiency in enhancing of clindamycin phosphate microemulsions would depend on the amount of the surfactant. As AOT in the system increased, the drug permeation increased. It is known that AOT is an anionic surfactant which may cause significant swelling of the stratum corneum, uncoiling and extending α-keratin helices and thereby opening up the protein-controlled polar pathway (Ghosh & Banga, 1993). As a result, the microemulsion systems of high amount of AOT would enhance more percutaneous permeation of clindamycin phosphate.

When compared between the different types of microemulsions at the same concentration of AOT (Figure 3), ME1 tended to enhance drug permeation higher than ME4 while ME2 had significantly greater effect than ME5 ($P < 0.05$). It can be seen that the o/w microemulsions enhanced clindamycin phosphate permeation greater than the w/o microemulsions. Many mechanisms of the permeation enhancement by microemulsions have been proposed (Peltola et al., 2003). Firstly, microemulsions could act as drug reservoirs where loaded-drug is released from the internal phase to the external phase and finally onto the skin. Secondly, microemulsion droplets might rupture on the surface of the stratum corneum and then release its content onto the skin. Thirdly, permeation of loaded-drug occurs directly from the droplets to the stratum corneum without microemulsion fusion to the stratum corneum. The last one would be the most likely mechanism of the skin permeation of clindamycin phosphate from our microemulsions. Owing to the

TABLE 3
Percentages of Drug Remaining in Clindamycin Phosphate Microemulsions[a] (n = 3)

	Percentages of Drug Remaining in Clindamycin Phosphate Microemulsions			
Formulation	Initial	1 Month	2 Months	3 Months
ME1	98.43 ± 0.71	99.01 ± 0.51	97.49 ± 0.76	95.22 ± 0.29
ME2	99.86 ± 0.42	97.46 ± 0.62	94.22 ± 0.54	92.59 ± 0.21
ME3	100.58 ± 0.69	98.19 ± 0.56	95.05 ± 0.80	92.34 ± 0.51
ME4	100.01 ± 0.52	96.36 ± 0.29	93.49 ± 0.38	90.29 ± 0.31
ME5	99.63 ± 0.31	97.00 ± 0.31	94.41 ± 0.45	91.08 ± 0.26

[a]Stored at 30 ± 1°C.

lipophilic nature of the stratum corneum, the loaded-drug in oil droplets of the o/w type might pervade into the epidermis sheet easier than the water droplets of the w/o type at the same surfactant concentration. Besides, an increase in the amount of the internal phase along with higher surfactant mixture resulted in higher drug permeation as seen in Figure 3 (ME3 > ME2 > ME1 and ME5 > ME4). This may be due to an increase in numerous small internal droplets which leads to increase surface area for the skin partition. In consequence, the droplets settle down to close contact with the epidermis providing high concentration gradient and increasing the drug permeation.

Stability of Microemulsions

During the stability study, no change in appearance of all clindamycin phosphate microemulsions was observed after storing at 30 ± 1°C for 1, 2, and 3 months, respectively. The pH values remained almost constant (4.72 – 5.52), and the microemulsions did not show phase separation or precipitation under centrifugal force. The percentages of drug remaining in each formulation were analyzed by HPLC, as presented in Table 3. The range of 90% and 99% of the initial clindamycin phosphate concentrations remained throughout the studied period. Assuming that the drug concentrations equal to or greater than 90% of the initial value indicate stability, therefore all studied clindamycin phosphate microemulsions were physically and chemically stable at 30 ± 1°C for at least 3 months.

CONCLUSION

The results from this study lead to the conclusion that the studied microemulsions were able to serve as efficient promoters for delivering clindamycin phosphate through human skin. The efficiency in enhancing the percutaneous permeation depended on the surfactant concentration in microemulsions. The microemulsion systems of higher AOT/1-butanol enhanced the drug permeation greater than the low surfactant systems. In addition, the o/w microemulsions showed higher drug permeation than the w/o type. Finally, all the studied microemulsions were physically and chemically stable at 30 ± 1°C for at least 3 months.

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