Kumar, T. C. A., David, G. F. X., Kumar, K., Umberkoman, R., Krishnamoorthy, M. S. (1976) A new approach with neuroendocrine pathways. In: Kumar, T. c. A. (ed.) Neuroendocrine Regulation of Fertility. Krager, Basel, pp. 314–322

Kumar, T. C. A., David, G. F. X., Sankaranarayanan, A., Puri, V., Sundram, K. R. (1982) Pharmacokinetics of progesterone after its administration to ovariectomized rhesus monkeys by injection, infusion, or nasal spraying. Proc. Natl. Acad. Sci. 79: 4185–4189

Pardridge, W. M. (1985) Strategies for drug delivery through the blood-brain barrier. In: Borchardt, R. T, Repta, A. J., Stella, V. J. (eds) Directed Drug Delivery, Humana Press, Clifton, pp 83–96

Pardridge, W. M. (1986) Receptor-mediated peptide transport through the blood-brain barrier. Endocr. Rev. 7: 314–330

Schurr, P. H., Maclaurin, R. L., Ingraham, F. D. (1953) Experimental studies on the circulation of the cerebrospinal fluid. J. Neurosurg. 10: 515–525

Su, K. S. E., Campanale, K. M., Mendelsohn, L. G., Kerschner, G. A., Gries, C. L. (1985) Nasal delivery of polypeptides I: Nasal absorption of enkephalins in rats. J. Pharm. Sci. 74: 394–398

Yoffey, J. M., Courtice, F. C. (1970) Lymphatics, Lymph and the Lymphomyeloid Complex. Academic Press, New York

J. Pharm. Pharmacol. 1991, 43: 451–454
Communicated December 20, 1990

Microemulsions as topical drug delivery vehicles: in-vitro transdermal studies of a model hydrophilic drug

D. W. OSBORNE, A. J. I. WARD*, K. J. O'NEILL†, *Drug Delivery R&D—Specialty Products, The Upjohn Company, Kalamazoo, MI, and * Department of Chemistry, Clarkson University, Potsdam, NY, USA*

Abstract—Microemulsions with a 58:42 weight ratio of dioctyl sodium sulphosuccinate:octanol and containing 15, 35, and 68% water have been tested for their ability to transport glucose across human cadaver skin. A flow-through multisample skin diffusion cell showed that both the 35 and 68% water microemulsions caused enhanced (approximately 30-fold) transport of glucose. No transport was discernible for the 15% water microemulsion. Differences in percutaneous glucose transport were shown to parallel differences in the diffusion of water within the microemulsion vehicles before application to the skin.

Microemulsions offer advantages over traditional creams and lotions as topical drug delivery formulations. Since microemulsions are thermodynamically stable, the properties of the formulation would not be dependent upon process, and the product will not phase-separate, provided temperature and pressure conditions remain reasonably constant. In addition to improved physical stability, microemulsions often function as "supersolvents" for certain compounds. Thus, these clear, fluid liquids may dramatically increase the solubility/solubilization of poorly soluble drugs. While microemulsions have significant potential as drug delivery vehicles, few well-characterized surfactant systems have been systematically studied (Florence 1981).

In a previous investigation (Osborne et al 1988a), the effect of microemulsion composition upon the in-vitro skin transport of water from a microemulsion system was investigated. The three microemulsions evaluated had a fixed weight ratio of surfactant to cosurfactant, and water concentrations of 15, 35, and 68 weight %. The study concluded that most of the water in the 15% microemulsion is bound to the surfactant headgroups and is not available for transport across the skin. Thus, the transport of water across the skin from that microemulsion is less than the transport for water itself. For the microemulsions with higher water content an approximately sixfold enhancement in water transport occurred. Pretreatment studies using the surfactant, cosurfactant, and surfactant/cosurfactant mixtures indicated that the enhancement in water penetration from the high water content microemulsions was a result of a synergistic effect between the surfactant, dioctyl sodium sulphosuccinate (DSS) and the cosurfactant octanol.

With this background established, it was considered important to complete and in-vitro transdermal transport study of a hydrophilic compound delivered from the same microemulsion vehicles. It was hypothesized that a hydrophilic drug would not be available for percutaneous transport from a microemulsion, unless water from the microemulsion is freely transported percutaneously. If this hypothesis is verified, then the topical formulator desiring to deliver hydrophilic drugs must assure 1) sufficient mobility of water within the microemulsion vehicle and 2) sufficient percutaneous transport of water across the skin barrier.

Correspondence: D. W. Osborne, Drug Delivery R&D—Specialty Products, The Upjohn Company, Kalamazoo, MI49001-0199, USA

† Present address: The Procter and Gamble Co., Egham, Surrey, UK.

Materials and methods

Dioctyl sodium sulphosuccinate USP (DSS) from American Cyanamid Company (Bridgewater, NJ) and 1-octanol (Aldrich 99%) were used as received. USP purified water was treated in a Millipore MILLI-Q filtration system before use, while tritiated water (5 mCi mL^{-1}) and D-[^{14}C(U)]glucose (3·7 mCi $mmol^{-1}$) were obtained from Amersham Corporation (Arlington Heights, IL). The in-vitro skin permeation studies were conducted on an apparatus as described by Holland et al (1984). This flow-through cell has a small-volume receiving chamber and a skin surface dosing area of 2 cm^2. Unlike the method described by Holland, 0·9% NaCl in distilled water was used as the receiving medium (flow rate 1·66 mL h^{-1}), and no attempts were made at maintaining skin viability. All dosing chambers were occluded with parafilm to prevent evaporation. Volumes of the donor phases were checked at the end of the experiment to assure that significant diffusion of the receiving fluid into the donor phase did not occur. Dermatomed human cadaver skin was obtained from a 57 year old caucasian female. The skin was frozen within 24 h of death and kept frozen until use. The skin was allowed to thaw gradually to room temperature (23°C) and thoroughly rinsed with purified water. After the skin had been mounted, water was used to maintain its hydration and to eliminate potential osmotic effects. This soaking was for a minimum of 4 h. The receiving medium was thermostated at 35°C.

Microemulsion samples for the skin permeation study were prepared by adding 25 μL [^{3}H]H_2O (50 mCi mL^{-1}) to a tared

reaction vial. The appropriate amount of water was then added and mixed, and 4 μCi D-[^{14}C(U)]glucose was added. The 58:42 by weight DSS:octanol solution was then added and periodically mixed over the next 24 to 72 h before use. Amounts were such to give a total sample weight of 2·5 g. A 0·5 mL dose of the microemulsion was placed on the skin surface and the saline receiving fluid was collected directly into the scintillation vials. After addition of Amersham PCS fluid, a dual label liquid scintillation counting study was completed using a Packard TRI-CARB 2000CA Liquid Scintillation Analyzer.

Infinite dose experiments using finite dose cells has been criticized recently (Addicks et al 1990). Since the Holland cell is designed for finite dose experiments, a study to determine the amount of donor phase necessary to be considered an infinite dose was completed. For the microemulsion studies, it was established that no difference existed in water transport profiles for 0·5 mL and 1·0 mL dosing, while a progressive decrease in transport after 10 h was seen for 0·25 mL and 0·1 mL dosing volumes. It was concluded that for this experimental design, a 0·5 mL occluded dose volume/2 cm^2 skin area could maintain a steady-state flux over the duration of the experiment.

Results

The phase behaviour for the water/octanol/DSS system (Fig. 1) is characterized by an extension toward the water corner for a narrow range of octanol/DDS mixtures. The 58:42 DSS:octanol weight ratio can incorporate greater than 70% water, making this system useful for evaluating the effect of microemulsion water content on transdermal flux. For this study, the advantages of the phase behaviour outweigh the practical limitation that octanol is too irritating to be used topically (Osborne et al 1988b). As indicated by the three phase regions at high water content, essentially pure water separates as the excess phase when greater than 84% water is added to the 58:42 DSS:octanol weight ratio. Thus, the 58:42 surfactant:cosurfactant ratio becomes saturated with water at the composition 84% water, 9% DSS and 7% octanol.

Fig. 2 shows in-vitro water transport from the three microemulsion compositions studied and the penetration of water. Transdermal flux values of 4·0, 1·6, 0·3 mg cm^{-2} h^{-1} resulted for the 68, 35 and 15% water microemulsions, respectively, while water alone gave a percutaneous flux value of 0·8 mg cm^{-2} h^{-1}.

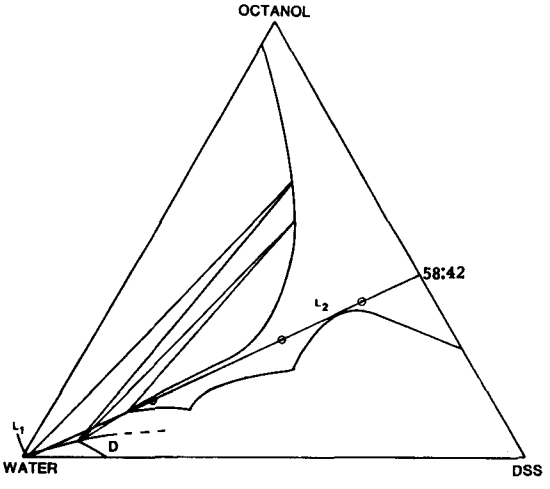

FIG. 1. Partial phase behaviour at 25°C of the system water/octanol/dioctyl sodium sulphosuccinate. Phases are labelled by: L_1 normal micellar; L_2 inverse micellar/microemulsion; and D lamellar liquid crystalline. Circled points on the 58:42 DSS/octanol constant ratio line correspond to the 15, 35, and 68% water compositions.

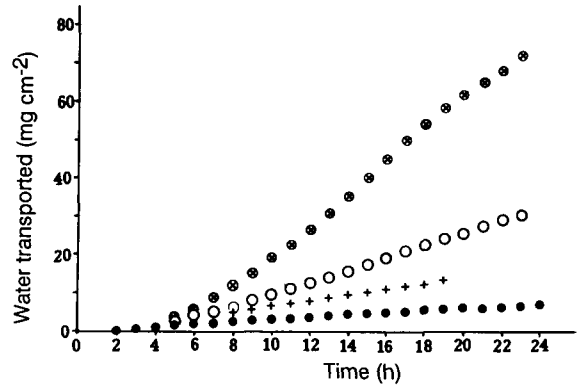

FIG. 2. In-vitro transdermal data for (+) pure water and the (●) 15, (○) 35 and (⊗) 68% water microemulsions formed from a 58:42 weight ratio of DSS/octanol. Cadaver skin was from the abdomen of a 57 year old Caucasian female using five replicates for each of the microemulsions and four replicates for pure water.

These water transport results were in good agreement with our previous study using fresh full-thickness skin (Bronaugh et al 1986) and with literature values for water alone (Barry 1983). Values for the graphically determined lag times and related parameters are given in Table 1. The longest lag of time of 5·1 h was for the 68% water content microemulsion. For the 35% preparation, the lag time increased to 4·2 h compared with the experimentally determined 2·1 h lag time for pure water. The 15% water microemulsion had a negative lag time upon extrapolation.

Table 1. In-vitro transdermal flux values and lag times for the microemulsion formed by addition of water to a 58:42 weight ratio of DSS/octanol.

	Flux (mg cm^{-2} h^{-1})	Lag time (h)	Corr*
Pure water	0·8	2·1	0·9997
15% water microemulsion	0·3	−1·8	0·9968
35% water microemulsion	1·6	4·2	0·9996
68% water microemulsion	4·0	5·1	0·9968

* Linear regression correlation coefficient based on data collected from 11–24 h for the 15 and 35% microemulsions, 13–24 h for the 68% water microemulsion and 8–19 h for water.

The transport of glucose across dermatomed cadaver skin is shown in Fig. 3. For the 15% water microemulsion (four replicates) containing 4 μCi [^{14}C]glucose, and pure water containing 4 μCi [^{14}C]glucose, counts above background were not discernible. Thus, transport of glucose from that microemulsion and from water was below the detection limits of the assay (0·03 nmol cm^{-2} h^{-1}). In contrast, the 35% (four replicates) and 68% (five replicates) water microemulsions at 20 h shared radioactivity approximately thirty-fold greater than background. As can be seen from the error bars for the 35% water microemulsion, the transport was variable and indistinguishable between the 35 and 68% water microemulsions.

Discussion

From Fig. 2, percutaneous water transport from water/octanol/DSS microemulsions can be seen to vary dramatically depending upon water content. Increasing the water content of the microemulsion from 15 to 35% resulted in a six-fold increase in water flux, while doubling from 35 to 68% resulted in an additional 2·5-fold increase in percutaneous water flux. As concluded by Osborne et al (1988a), DSS/octanol is acting to increase the permeability of the stratum corneum. For the 35 and

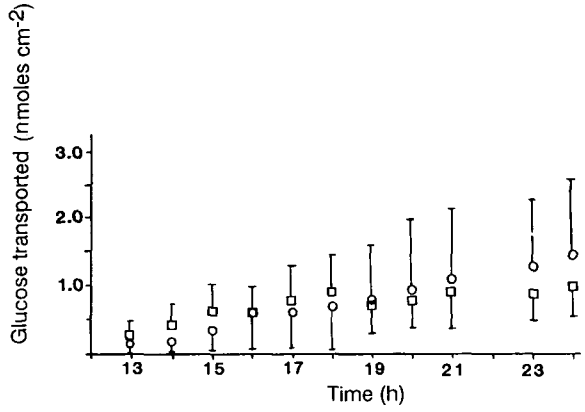

FIG. 3. In-vitro transdermal data for the 35 (□) and 68% water (○) microemulsions. Each point gives the amount of D-glucose recovered at the hour specified, i.e. this is not a cumulative plot. Error bars are the standard deviations of four replicates (□, 35% water) and five replicates (○, 68% water) using human cadaver skin from the same donor.

68% water microemulsions, the increased water transport compared with that of pure water is explained by the ability of DSS/octanol to increase stratum corneum permeability. For an ideal solution, doubling the concentration of a dissolved penetrant will double the transport of the penetrant. The approximate doubling of the water concentration from 35 to 68% results in a 2·5-fold increase in flux. This is in dramatic contrast to the sixfold decrease in water flux after the approximate halving of water concentration from 35 to 15% water and is attributed to most of the water in the 15% microemulsion being bound to the surfactant headgroups (Osborne et al 1988a), and unavailable for transport across the skin. The strong hydrogen bonds characteristic of these waters of hydration do not behave ideally, thus hindering the release of water from the 15% water microemulsion. The percutaneous water flux from the 15% water microemulsion is threefold less than the percutaneous flux of water, despite the increase in stratum corneum permeability brought about by DSS/octanol. So, for this microemulsion system, initial addition of water to the surfactant/cosurfactant mixture is used for solvation of the surfactant headgroup and is unavailable for percutaneous transport. Upon subsequent addition, free water, i.e. water in excess of that required for headgroup hydration, becomes available within the system, and increases in percutaneous water flux with increased water concentration exhibit virtually "ideal" behaviour.

As hypothesized, glucose was not available for percutaneous transport from the microemulsion, unless water was freely transported percutaneously. Glucose delivery from the 35 and 68% water microemulsions was approximately thirty-fold compared with pure water containing an equal trace amount of glucose. However, the percutaneous transport is highly variable and characterized by long lag times. Both effects have been noted previously for transdermal glucose transport (Ackermann & Flynn 1987). For these microemulsions the presence of free water appears to be essential for glucose to be freely available for partitioning into the skin. These results indicate a simple mechanism. The trace amount of glucose resides within the hydrophilic portion of the microemulsion over the entire water concentration range. For the lowest water content microemulsions, essentially all the water is bound to the amphiphiles and, therefore, glucose is in a bound water environment and generally unavailable for partitioning into the skin. For the higher water content microemulsions, free water exists which contains glucose freely available for partitioning into the skin. Presumably, the presence of free water would be necessary for the significant transport of any hydrophilic drug. Furthermore, the enhanced transport of glucose from the higher water content microemulsions can be explained by the enhancer effects of the DSS/octanol on the skin. Four μCi [^{14}C]glucose corresponds to 0·008% glucose in the formulations. The saturation value of glucose in these microemulsions is difficult to determine exactly because of substantial thickening of the liquid between 10 and 30% glucose. However, for each of the microemulsions studied, greater than 10% glucose could be dissolved/solubilized. Since the amount of glucose added was less than one thousandth the glucose saturation value of the microemulsion, differences in thermodynamic driving force due to different degrees of saturation have been minimized. While a more dilute concentration might approach infinitely dilute conditions, the corresponding loss in assay sensitivity seemed unacceptable.

As detailed above, the steady-state flux values of water and glucose are readily explained by the competing mechanisms of DSS/octanol increased stratum corneum permeability and the absence of free water until surfactant headgroup solvation is completed. Alone, these explanations cannot account for the negative lag time seen for the 15% water microemulsion. While the low slope of the plot in Fig. 2 for the 15% water microemulsion will increase the error of the intercept measurement, experimental or graphical error is not sufficient to explain the observed $-1{\cdot}8$ h lag time. Lag time, τ, is conventionally developed in terms of the passive diffusion equation written as:

$$Q_t = \frac{DP\Delta C_v}{h}(t-\tau)$$

where Q_t is the total amount absorbed in time t, D is the solute diffusion coefficient, ΔC_v is the difference in the solute concentration between the vehicle and tissue, P is the solute partition coefficient between vehicle and skin and h is the thickness of the stratum corneum-vehicle interfacial region. The lag time has been shown to be equal to $h^2/6D$. Generally, the value of h has been taken as the thickness of the stratum corneum using the measured lag times to obtain values for the diffusion coefficients. Such a simple passive description of the diffusion barrier is limited because the active interaction of components at the vehicle-stratum corneum interface will effectively alter the value of h.

The negative lag time for the 15% water microemulsion could result from a significant decrease in h over the initial dosing period. Note that the water flux values for this study, in which the skin was soaked with water for a minimum of 4 h before

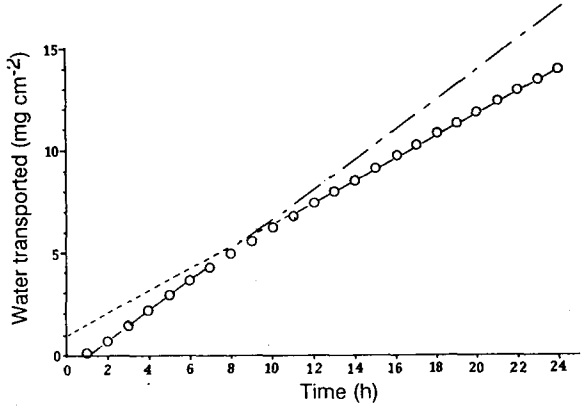

FIG. 4. Detailed plot of lag time extrapolations for the 15% water microemulsion. The dashed (-----) line is the extrapolation of the 11–24 h points that give a lag time of 1·8 h. The broken line (— –) is the extrapolation of the 3–6 h collection points that give a lag time of 0·9 h.

dosing are in good agreement with water flux values found by Osborne et al (1988a) with skin soaked in chilled water for 52 h. This agreement can be attributed to the cadaver skin used in the present study being significantly hydrated before being mounted in the cell. Hydrated stratum corneum should be significantly swollen. Application of the low water microemulsion causes water to be pulled from the stratum corneum into the donor liquid. During this dehydration of stratum corneum its thickness continues to decrease until an equilibrium gives rise to a "steady-state" tissue that is less hydrated and less permeable to water and glucose than at the start of the experiment. This description is supported by a calculated lag time of 0·9 h ($r^2 = 0.999$) based on the 3–6 h collection period (Fig. 4). Note that for a constant diffusion coefficient of $4{\cdot}2 \times 10^{10}$ cm^2 s, a decrease in stratum corneum thickness from 43 μm to 23 μm results in a 1·5 h difference in lag time as calculated by the $h^2/6D$ relationship.

Conclusions

Based upon results for water and glucose, it was found that topical delivery from the microemulsions studied was highly variable and extremely dependent upon composition. While each of the three microemulsions evaluated had the same ratio of surfactant to cosurfactant, differences in water content caused the in-vitro percutaneous transport of water and glucose to vary fifteenfold and greater than thirtyfold, respectively. As hypothesized, glucose transport was achievable only from microemulsions that had water in excess of that required for amphiphile hydration as indicated by flux values greater than the steady-state flux value for pure water. Thus, while microemulsions may provide topical formulations with increased physical stability and superior drug solubility/solubilization, microemulsion compositions must be carefully optimized to achieve maximum percutaneous transport.

References

Ackermann, C., Flynn, G. L. (1987) Ether-water partitioning and permeability through nude mouse skin in vitro. I. Urea, thiourea, glycerol and glucose. Int. J. Pharm. 36: 61–66

Addicks, W. J., Flynn, F. L., Weiner, N. D., Curl, R. L. (1990) Drug delivery from topical formulations: theoretical prediction and experimental assessment In: Osborne, D. W., Amann, A. H. (eds) Topical Drug Delivery Formulations, Marcel Dekker, Inc., New York, pp. 221–244

Barry, B. W. (1983) Dermatological Formulations. Marcel Dekker, Inc., New York, p. 110

Bronaugh, R. L., Stewart, R. F., Simon, M. (1986) Methods for in vitro percutaneous absorption studies VII: use of excised human skin. J. Pharm. Sci. 75: 1094–1097

Florence, A. T. (1981) Drug solubilization surfactant systems. Drugs Pharm. Sci. 12: 15–89

Holland, J. M., Kao, J. Y., Whitaker, M. J. (1984) A multisample apparatus for kinetic evaluation of skin penetration in vitro: the influence of viability and metabolic status of the skin. Toxicol. Appl. Pharmacol. 72: 72–280

Osborne, D. W., Ward, A. J. I., O'Neill, K. J. (1988a) Microemulsions as topical drug delivery vehicles. I. Characterization of a model system. Drug Dev. Indust. Pharmacy 14: 1203–1219

Osborne, D. W., Middleton, C. A., Rogers, R. L. (1988b) Alcohol free microemulsions. J. Disp. Sci. Tech. 9: 415–423

J. Pharm. Pharmacol. 1991, 43: 454–455
Communicated July 7, 1990

Letter to the Editor

Effect of heparin sodium on limulus amoebocyte lysate preparations from two manufacturers

N. V. NAIDU, I. J. KHALIFA, V. R. NALLARI, H. I. RAUT, *Drug Control and Registration Centre, Ministry of Health, P.O. Box 22575, Zip. Code. 13086, Safat, Kuwait*

The limulus amoebocyte lysate (LAL) test is included in the USP XXI (1985) as a bacterial endotoxin test (BET). However, for the test to be valid for end-product evaluation of any preparation the properties of the product itself should not invalidate the endotoxin-lysate reaction (USFDA 1987).

Inhibition of the limulus reaction by the heparin preparations was first reported by Sullivan & Watson (1975), who found that the inhibition could be overcome by the addition of calcium and sodium salts. Ronneberger (1982) found inhibition of gel formation with 50 units mL^{-1} of heparin. Heparin sodium tested at 1000 units mL^{-1} also inhibited three out of five lysates from different manufacturers (Twohy et al 1983).

During a routine procedure in this laboratory to validate the LAL test for detection of pyrogens in commercially available heparin preparations, it was found that heparin produced different effects on lysate preparations from two manufacturers (Whittaker M. A. Bioproducts (Pyrogent) and Associates of Cape Cod Inc. (Pyrotell)). While levels greater than 2·5 units mL^{-1} inhibited the Pyrogent brand lysate, no inhibitory effects were observed up to 100 units mL^{-1} tested with Pyrotell brand lysate. An attempt was made to see if the inhibitory effects of heparin on Pyrogent brand lysate could be overcome by the addition of calcium and sodium salts to the endotoxin-lysate mixture in the laboratory. The results are presented.

Control Standard Endotoxin, Lot No. L02087 obtained from Whittaker M. A. Bioproducts, Strain *Escherichia coli* 055:B5 with a defined activity of 9 EU ng^{-1} in terms of US Reference Standard Endotoxin (RSE), EC-5, was added to the heparin solutions. Details of the lysate brands used, along with their batch numbers and labelled sensitivities, are shown in the footnote to Table 1.

Serial dilutions of endotoxins bracketing the sensitivity of the lysate used were prepared in sterile water for injection (SWI) containing specified concentrations of heparin sodium. Similar dilutions of endotoxins containing no heparin formed the controls for confirmation of the labelled sensitivities of the lysates. A sample of each diluted solution (0·1 mL) was distributed into 10 × 75 mm borosilicate test tubes. A portion of the reconstituted lysate (0·1 mL) was then added to each tube

Correspondence: I. J. Khalifa, Director, Drug Control and Registration Centre, Ministry of Health, P.O. Box 22575, Zip. Code. 13086, Safat, Kuwait.