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Delivery of a hydrophilic solute through the skin from novel microemulsion systems

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Abstract

Five microemulsions were prepared with a mean radius of the internal phase droplets varying from 10 to 70 nm. The microemulsions were evaluated for their ability to deliver a model hydrophilic solute (sucrose) across hairless mouse skin in vitro. Maximum sucrose fluxes, following application of the different microemulsions for 9 h, were similar and were about an order of magnitude greater than that from a 20 mM sucrose aqueous solution. The five (unloaded) formulations and three controls (water, propylene glycol and 5% oleic acid in propylene glycol) were applied for 3 h to the ventral forearm of six volunteers. Transepidermal water loss (TEWL) and relative skin blood flow (SBF) were measured immediately after removing the formulations and repeatedly over a further 3 hour period. SBF increased significantly only after application of the oleic acid/propylene glycol positive control; for all other treatments, SBF remained at the pretreatment value. Immediately after removing all the formulations, TEWL was elevated. However, these values quickly recovered to the pretreatment control except in the case of oleic acid/propylene glycol. Overall, this preliminary study indicates that microemulsion formulations can be used to improve the delivery of hydrophilic solutes while eliciting insignificant effects on human skin in vivo.

Keywords: Microemulsions; Penetration enhancement; Relative skin blood flow; Sucrose; Transdermal delivery; Transepidermal water loss

1. Introduction

Microemulsions and related systems represent pharmaceutically versatile formulations for various applications, including drug delivery to and through the skin [1]. These systems can increase the transdermal delivery of a compound by different mechanisms. First, a large amount of drug can be included in the formulation due to the high solubilization power. Second, an increase in the transdermal flux can be expected in that the thermodynamic activity of the drug in the microemulsion can be modified to favour partitioning into the stratum corneum. Third, the surfactants in the microemulsion may reduce the diffusional barrier of the stratum corneum. This last effect can be more or less important depending on the nature of the tensioactives.

The long-term objective of this project is to evaluate the potential of microemulsions to effectively deliver drugs transdermally and to identify those factors influencing the transport. As a starting point, we have

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chosen a highly hydrophilic compound, sucrose, to evaluate the influence of phase composition on transdermal delivery. It has been reported that the delivery of glucose from a microemulsion was highly dependent upon the aqueous content of the system [2]. In this paper we have followed sucrose delivery from microemulsions, formulated with non-irritant surfactant and cosurfactant constituents, in which the aqueous phase comprised between 14 and 67% of the system. In particular, we addressed the following questions: (i) can the delivery of sucrose be improved through the skin by incorporation into a microemulsion?; (ii) does the structure and composition of the microemulsion modify the delivery?; and (iii) are the formulated microemulsions 'well-tolerated' in vivo?

2. Materials and methods

2.1. Preparation of microemulsions

Labrasol® (L, polyethylene glycol-8 caprylate/caprate) and Plurol Isostearique® (P, polyglyceryl isostearate) (Gattefossé, Lyon, France) were weighed into a glass vial and the appropriate amount of ethyl oleate (EO) (Aldrich Chemical, Milwaukee, WI) was added (Table 1). The aqueous phase (154 mM NaCl) was then introduced. Sucrose was incorporated into the aqueous phase at various concentrations (30 to 143 mM) so that the final, nominal level of sucrose in each of the microemulsions studied was 20 mM. To facilitate transdermal flux measurements, the aqueous solution was 'spiked' with the ^{14}C-radiolabeled sucrose (NEN Research Products, Wilmington, DE). The mixtures were left overnight to allow the microemulsions to form. Microemulsions for the in vivo experiments were prepared identically except that no sucrose was used.

2.2. Microemulsion characterization

The microemulsions were all clear, stable liquid formulations. The size of the dispersed phase of the undiluted microemulsions (both with and without sucrose) was measured at 25°C by dynamic laser light scattering (ALV-5000, ALV GmbH, Langen, Germany). The scattering intensity data were obtained from pre-filtered (0.45 μm) microemulsions at angles of 60, 90 and 120°. The scattering intensity data were analyzed by a digital correlator, and the correlation functions fitted by the method of 'inverse Laplace transformation' and CONTIN [3,4]. Based on the earlier characterization of the microemulsion system studied [5], and the observed conductivity values reported here (see below), it was deduced that microemulsions 2 and 4 were W/O, while microemulsions 1, 3 and 5 were O/W (thus, the solvent refraction indices and viscosities were those of ethyl oleate and water, respectively). To characterize the specific conductivity of the systems, a Wheatstone bridge (Databridge 452, Hungtingdon, UK) was used. Measurements were made using grey platinized electrodes at three different frequencies (100 Hz, 1 KHz and 10 KHz) over the temperature range 30–40°C at 2°C intervals. Data were transferred on-line to a computer (IBM-XT, Finland) and conductivity obtained by extrapolation to infinite frequency. Temperature of the conductivity cell was controlled ($\pm$0.01) by a circulating water bath (Barrington, Grant-LTD6, Cambridge, UK) which drives water through an spiral around the cell.

2.3. Transdermal delivery experiments

Freshly excised hairless mouse skin (Simonsen Laboratories, Gilroy, CA) was clamped between the donor and receptor chambers of vertical flow-through diffusion cells (3.14 cm^2; LGA, Berkeley, CA). The degassed receptor fluid (133 mM NaCl buffered at pH 7.4 with 25 mM HEPES) was continuously perfused at 3 ml/h (Manostat, NY). Donor phase volume was 3 ml. Control experiments used, as donor solutions, 20 and 143 mM sucrose in 154 mM NaCl (aq.). Hourly samples were collected on a fraction collector (ISCO, Lincoln, NE). The samples were mixed with an appropiate volume of scintillation fluid (Ready-gel, Beckman Instruments, Irvine, CA) and analyzed for ^{14}C-radiolabel in a liquid scintillation counter (Beckman Instruments, Irvine, CA). The measured dpm were transformed into molar flux by taking into account the appropriate correction necessary for flow rate and receiver phase volume [6]. At least five replicates were performed for each formulation. All animal procedures were approved by the UCSF Committee on Animal Research.

Table 1
Microemulsion composition (in vol.%)

ME	Aqueous solution		Ethyl oleate	Labrasol: plurol (3:1)
	Sucrose mM	*NaCl mM*		
ME.1	67.5 *30*	*154*	7.5	25
ME.2	14 *143*	*154*	49	37
ME.3	42 *48*	*154*	14	44
ME.4	19 *105*	*154*	37	44
ME.5	31 *64*	*154*	25	44

2.4. In vivo studies

To test the acceptability of the microemulsions as topical vehicles, the five microemulsions and three controls (water, propylene glycol and 5% oleic acid in propylene glycol) were separately applied for 3 h to the ventral forearm of six volunteers. All in vivo procedures were approved by the UCSF Committee on Human Research. The formulations were applied to the skin via a simple dermatological dressing (Soft-Wick, iv sponges®, Johnson and Johnson, Arlington TX) (6.25 cm^2), which was covered by an occlusive film, and then firmly attached to the forearm by adhesive tape (Tegaderm®, 3M Co., St. Paul, MN). Transepidermal water loss (TEWL) and relative skin blood flow (SBF) were measured before application, immediately after removing the formulation, and repeatedly the next 3 h. TEWL was determined using an evaporimeter EP 1C and 1D (Servomed USA, Broomall, PA, USA); SBF measurements employed a laser Doppler flowmeter (Periflux, Perimed, KB, Sweden). Results were expressed as Normalized-TEWL and Normalized-SBF, i.e. the ratio between any given measure and the original (before application) value.

2.5. Statistics

The data were analyzed statistically by 1- and 2-factor ANOVA and the Fisher PLSD test (level of significance = 0.05).

3. Results

The microemulsions were all clear, stable liquid formulations. The light scattering experiments revealed that the droplet radius for all the microemulsions fell in the range of 9–40 nm (Table 2). Asymmetry and agglomerates were apparently present in some systems. The presence or absence of sucrose, in most cases, did not significantly affect the size of the internal droplets. Conductivity values increased for the systems that contained a higher percentage of water and also increased slightly with temperature (Fig. 1).

Sucrose flux from aqueous solution reached an apparently constant value after 5–6 h. For the microemulsions, however, the flux continuously increased throughout the experiment (Fig. 2). The maximum sucrose fluxes, delivered from the microemulsions across the skin, following 9 h of application were somewhat independent of the formulation (Fig. 3). This conclusion is independent of the time chosen for comparison. The transport rate at 9 h from the microemulsion were about an order of magnitude greater than the flux of sucrose from a 20 mM aqueous solution. Statistical treatment showed that, while sucrose delivery from the microemulsions showed no significant dependence upon the formulation, all microemulsions were significantly more effective vehicles than water (sucrose 20 mM) alone. Indeed, three of the microemulsions delivered more sucrose than an aqueous solution at 143 mM.

Table 2
Size of the dispersed phase of the microemulsions (ME) with and without sucrose (S), measured by laser light scattering at three different angles

ME	Radius[a] (nm)		
	60°	90°	120°
ME.1	9, 65	10, 75	8, 120
ME.1+S	10, 37	13, 119	12, 104
ME.2	12, 49	51	49
ME.2+S	32	41	42
ME.3	11	4, 19	4, 21
ME.3+S	3, 12	5, 21	5, 21
ME.4	26	27	29
ME.4+S	25	27	25
ME.5	9, 22	5, 21	7, 24
ME.5+S	7, 25	7, 28	6, 27

[a] Where two values are listed, the first correponds to the size of the individual microemulsion droplets, while the second is that of the aggregates formed by these smaller structures.

For the purposes of comparison, the absolute TEWL and SBF values were normalized with respect to the corresponding pre-treatment control levels. The variation of these normalized parameters, as a function of time post-application of the various formulations, are shown in Figs. 4 and 5 (TEWL and SBF, respectively). Immediately after removal of all the vehicles, TEWL was elevated, presumably due to hydration of the skin caused by occlusion. At 1 and 3 h post-removal of the

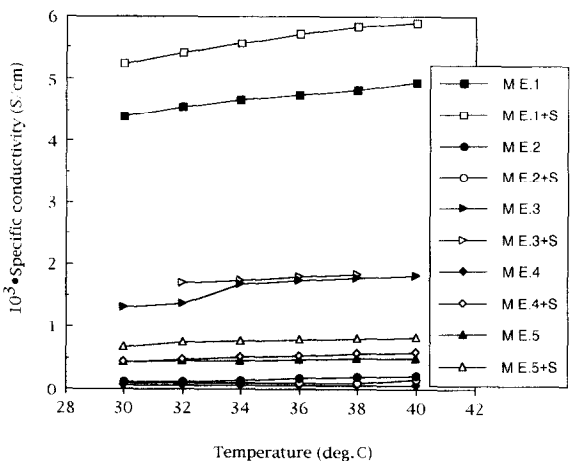

Fig. 1. Specific conductivity values of the different formulations alone, and loaded with sucrose (+S), as a function of temperature.

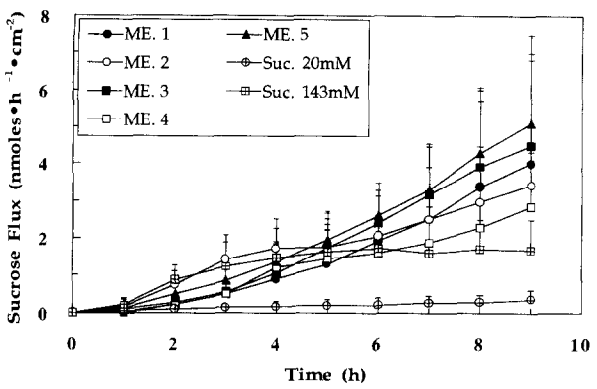

Fig. 2. Sucrose fluxes through hairless mouse skin in vitro from aqueous solutions and five microemulsion formulations.

formulations, however, 5% oleic acid in propylene glycol (O.A.) was the only vehicle that caused TEWL to remain significantly elevated relative to the other treatments (i.e. the five microemulsions, P.G. alone, and water). The SBF data reinforced the conclusion that, while the microemulsions were no more perturbing to the skin than either water or P.G., O.A. caused significant erythema which persisted for at least 3 h post-treatment.

4. Discussion

The microemulsion system studied in this work has been previously characterized in our laboratoty [5]. An important feature of the system is its composition of putative non-irritant tensioactives and cosurfactants which may be particularly suitable for topical and transdermal drug delivery. The literature addressing cutaneous application of microemulsion systems has been reviewed recently [7] and it is clear that, while these formulations have unique and possibly very advantageous properties, their systematic evaluation (both with respect to physical chemistry and drug delivery) remains to be accomplished. It was therefore an important objective of our study to propose and perform

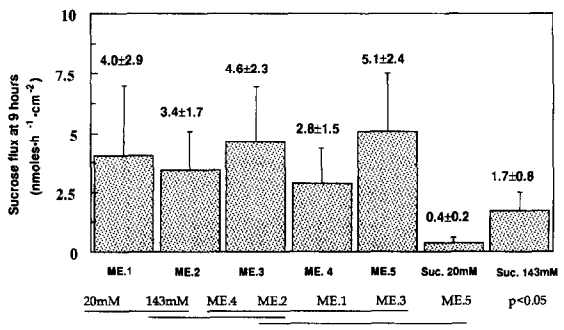

Fig. 3. Sucrose fluxes at 9 h. The lower portion of the figure ranks the average delivery rates and formulations, which deliver statistically indistinguishable amounts of sucrose, are underlined together.

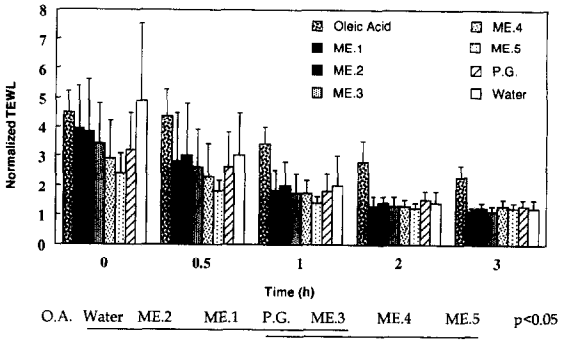

Fig. 4. Normalized TEWL values, as a function of time post-application of the various formulations. The lower portion of the figure shows the result of the two-way ANOVA for the variables formulation and time. Formulations, which are not statistically distinguishable, are underlined together (results for the variable time are not shown).

experimental procedures designed to address this crucial issue.

The range of microemulsion compositions used was limited by the corresponding region of the NaCl/Plurol + Labrasol/Ethyl Oleate phase diagram [5]. The composition explored utilized the minimum percentages of tensioactives while maximizing the range of water-to-oil proportions.

The term 'microemulsion' is used to describe a variety of physical systems, including swollen micelles, o/w and w/o microemulsions, bicontinuous structures, etc. Assigning the correct structure to a particular system is complex and requires more than one characterization technique [8]. Because of the high concentration of the dispersed phase in the microemulsions, interaction between droplets cannot be excluded. The light scattering results (Table 2) lend support to this conclusion. Equally, the data in Fig. 1 show that addition of sucrose to certain microemulsions can (somewhat surprisingly) cause their conductivity to increase. It is pausible that these changes reflect either significant alterations in particle size(s) present and/or the appear

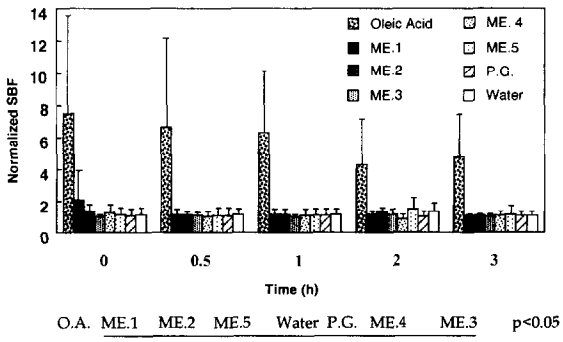

Fig. 5. Normalized SBF values, as a function of time post-application of the various formulations. The lower portion of the figure shows the result of the two-way ANOVA for the variables formulation and time. Formulations, which are not statistically distinguishable, are underlined together (results for the variable time are not shown).

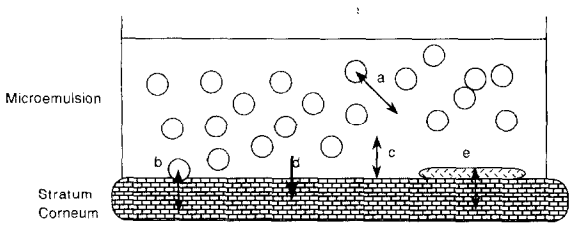

Fig. 6. Schematic diagram of the possible events involved in transdermal drug delivery from a microemulsion. Partitioning of the incorporated drug can take place from one or more of the different phases of the microemulsion (a, b, c). Components of the vehicle can diffuse into the SC and modify the permeability of the barrier (d). Microemulsion structure can be lost upon contact with the SC such that delivery may occur from a completely different physical form (e) that now includes, for example, lipid components from the skin.

ance of bicontinuous structures. To unequivocally unravel these subtle effects clearly requires more sophisticated and microscopic techniques.

Sucrose flux from the microemulsions was moderately higher than that from aqueous solution, but the level of enhancement achieved is smaller than that observed with iontophoresis [9]. The uncertainty about the structure of the systems makes it difficult to link structure with flux. In general, the microemulsions containing a higher percentage of aqueous phase delivered sucrose better. In fact, the largest delivery was achieved with the system containing the three constituents at essentially similar percentage volumes (31:25:44).

Fig. 6 presents a schematic diagram of the variety of possible events involved in topical delivery from a microemulsion formulation. Firstly, there are different partitioning processes occurring: between the internal and external phases of the microemulsion, and between either the internal or the external phase of the microemulsion and the skin (steps a, b, c). Drug transport may be controlled by any of these processes, and the thermodynamic driving force for release will reflect the relative activities of the drug in the different phases. Next, it is conceivable (and often likely) that one or more component of the microemulsion itself enters the skin, and interacts in some way with the membrane to alter the barrier properties (step (d)), i.e. to increase permeability. Given that sucrose flux from the microemulsions increases continually over the course of the permeation experiments, this scenario may be quite important. Finally, there is the additional possibility that constituents of the skin barrier (e.g. lipids) are extracted by the microemulsion leading to a new physical entity (perhaps comprising only a very thin layer in contact with the skin surface) from which some part of drug delivery now takes place (step e). If this represents a significant fraction of the mechanism of drug absorption, then it will be important to evolve techniques to characterize this process during formulation development.

Finally, with respect to the human experiments, changes in insensible water loss and is the perfusion of the dermal microcirculation were selected as markers of barrier function based upon their reported application as such in numerous earlier studies (e.g. [10–13]). The in vivo measurements of TEWL and SBF indicate that the microemulsions are well-tolerated. This result is quite interesting given the high content of surfactants and ethyl oleate in some of the formulations. In this sense, it is important to recall the different characteristics of hairless mouse and human skin. Both the enhancement effect in human skin and the absence of irritant effects with longer and repeated application times will need to be demonstrated. Nevertheless, the microemulsions did appear to be well-tolerated in comparison to a simpler formulation of the frequently used penetration enhancer, oleic acid.

We conclude that microemulsion delivery systems for topical application can be formulated which: (a) provide enhanced delivery of an otherwise very impermeable compound; and (b) perturb the skin to a relatively small extent. The generality of these observations, and their mechanistic basis, remain subjects for continued and more detailed investigation.

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