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Bicontinuous sucrose ester microemulsion: a new vehicle for topical delivery of niflumic acid

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Abstract

The bicontinuous sucrose ester based microemulsion microstructure was characterised by a freeze fracture electron micrograph (FFEM) technique. The relationship between the microstructure and the efficacy of the microemulsion (ME) as a drug carrier system was investigated. The bioavailability of niflumic acid, a potent anti-inflammatory drug, incorporated at different concentrations in the microemulsion vehicle was investigated in vivo and compared with Nifluril® ointment (3%), a commercially available form. The methyl nicotinate model was used to induce inflammation. Following topical application of methyl nicotinate, various niflumic acid forms were immediately applied to the skin. The vascular response to methyl nicotinate on the treated areas was monitored by a Laser Doppler Flowmetry technique. The results exhibit the performance of the microemulsion vehicle as a niflumic acid carrier compared to the marketed form. The 1% niflumic acid microemulsion is as efficient as the 3% niflumic acid ointment.

Keywords: Bicontinuous structures; Microemulsions; Methyl nicotinate; Sucrose esters

1. Introduction

The topical route for drug delivery has attracted great interest.

The design and development of new drug delivery systems in order to enhance their efficacy is an ongoing process in pharmaceutical research.

Thus, new approaches of formulation are needed. Microemulsions (ME) are well known to improve absorption and bioavailability of many compounds (Bhargava et al., 1987). Some advantages offered by microemulsions include improvement in drug solubilisation, enhancement of bioavailability, protection of the drug against the environment. Their manufacture needs little energy input and they have a long shelf life (Constantinides, 1995). However, few microemulsion

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Table 1
Assessed formulations

Ingredients	Suppliers	ME 1 %(w/w)	ME 0.6 %(w/w)	ME 0.3 %(w/w)	Placebo ME %(w/w)
Sucrose monolaurate	Kagaku Foods (Tokyo)	3.24	3.24	3.24	3.24
Sucrose dilaurate	Kagaku Foods (Tokyo)	14.76	14.76	14.76	14.76
Medium chain alcohol	Gattefossé (St Priest France)	12	12	12	12
Octyl octanoate	Dragoco	45	45	45	45
Niflumic acid	Upsa	1	0.6	0.3	0
Purified water		24	24.4	24.7	25

systems in marketed products have been identified and the design of effective topical drugs formulated in pharmaceutically suitable microemulsion vehicles still remains a challenge.

This study was undertaken first to assess the presence of a bicontinuous structure in a ME system and secondly to investigate the influence of a bicontinuous microemulsion vehicle on the topical bioavailability of niflumic acid, a non steroidal anti-inflammatory drug (NSAID), which has been proved to possess percutaneous pharmacological activity (Poelman et al., 1989, 1991). In addition, we studied whether its anti-inflammatory effect is enhanced over commercial formulations. Topically applied nicotinates are widely used for their ability to provoke local cutaneous vasodilation (Guy et al., 1983). The prostaglandins are involved in the vascular response to methyl nicotinate (MN), and therefore induce skin inflammation (Wilkin et al., 1985). The decrease of inflammation, was recorded after application of the various topical niflumic acid formulations by laser doppler velocimetry (LDV).

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

The assessed formulations containing increasing percentages of niflumic acid are listed in Table 1. Saturation is reached in ME 1. MN was supplied by Janssen Chemicals (Beerse, Belgium). A 50 mM aqueous nicotinate solution was prepared. Nifluril® ointment 3% is marketed by UPSA Laboratories.

2.1.2. Test subjects

Volunteers were 12 healthy women (age range 24–35) with no history of dermatological disease. All gave written informed consent. They were asked not to ingest or apply to the skin any anti-inflammatory drug for one week before the study.

2.2. Methods

2.2.1. Freeze Fracture Electron Microscopy (FFEM) technique

The procedure comprises four steps: sample preparation, freezing of the specimen, fracturing and replication and finally microscopic investigation of the replicas.

The samples are prepared by weight and homogenised by stirring with Teflon coated magnet at 25°C. After few seconds, the samples are rapidly transferred into liquid propane at −196°C. The cooling rate is of the order of 2000°C/s.

For fracturing, the samples are clamped under liquid nitrogen inside the vacuum chamber of the freeze etching apparatus (Balzers BAF 400 Freeze Etching Apparatus). The preparation (support and sample) is warmed by temperature control, up to −150°C. Fracturing is then achieved by displacing a microtome arm cooled by liquid nitrogen. The now exposed fracture face is immedi-

ately shadowed unidirectionally by Pt-C. The specimens are washed in THF acid and the replicas are then investigated with a transmission electron microscope with tilt device for stereoimage (JEOL 100SX transmission electron microscope).

2.2.2. In vivo experiment

Six areas were delineated on the ventral surface of the forearm. The MN solution was applied at $t = 0$ with a gloved finger and allowed to dry. Then, 2 mg/cm^2 of the different formulations (Table 1) were randomly applied and were spread uniformly for 1 min. Then, the anti-inflammatory activity was monitored over 35 min, on each zone, versus placebo ME and untreated skin (US). The paired bilateral Student's t-test was used to compare the following parameters involved in the blood flow response:

1. The AUC (cm^2).
2. The percentage of inhibition of the induced MN inflammatory reaction calculated from the following equation.

$$\text{Inhibition (\%)} = \frac{\text{AUC(PC)} - \text{AUC(D)}}{\text{AUC(PC)}} \times 100$$

where AUC(PC) corresponds to the area under the response time curve on the placebo ME site and AUC(D) to the area under the response time curve on the drug treated site.

3. The lag time before the vasodilation decrease, which depends upon the bioavailability of the NSAID in each vehicle.
4. The slope of the decaying inflammation process, which assesses the vehicle effect.

3. Results

3.1. Microemulsion microstructure

Figs. 1 and 2 illustrate the microscopic features of the microemulsion vehicle and of the 1% niflumic acid microemulsion. The freeze fracture technique allows observation of a specific structure, which is consistent with the picture of bicontinuous phases described by Jahn and Strey (1988). The intertwined domains of water and oil are evident and the presence of niflumic acid in the sample does not change the irregular shaped bicontinuous microemulsion.

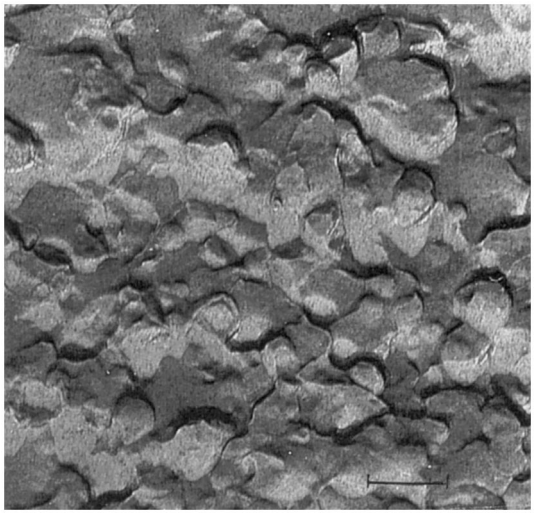

Fig. 1. Image of the freeze fractured ME vehicle. Bar = 200 nm. See the labyrinthic network of the aqueous and lipophilic domains.

3.2. In vivo experiments

(1) The high AUC value calculated for the untreated skin site (see Fig. 3) (251.4 ± 70 cm^2) confirms the percutaneous absorption of the nicotinic ester (vasodilatory effect).

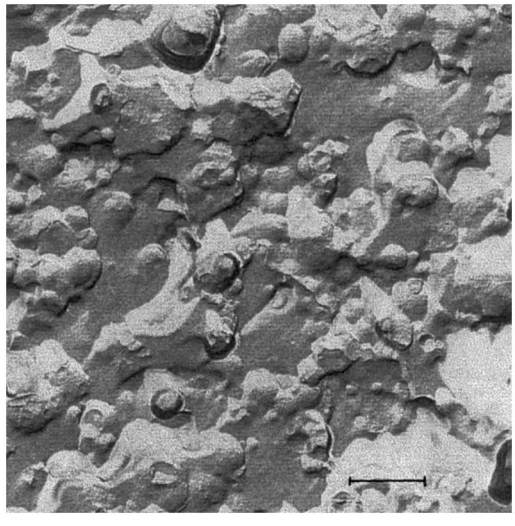

Fig. 2. Image of the freeze fractured ME 1. Bar = 200 nm.

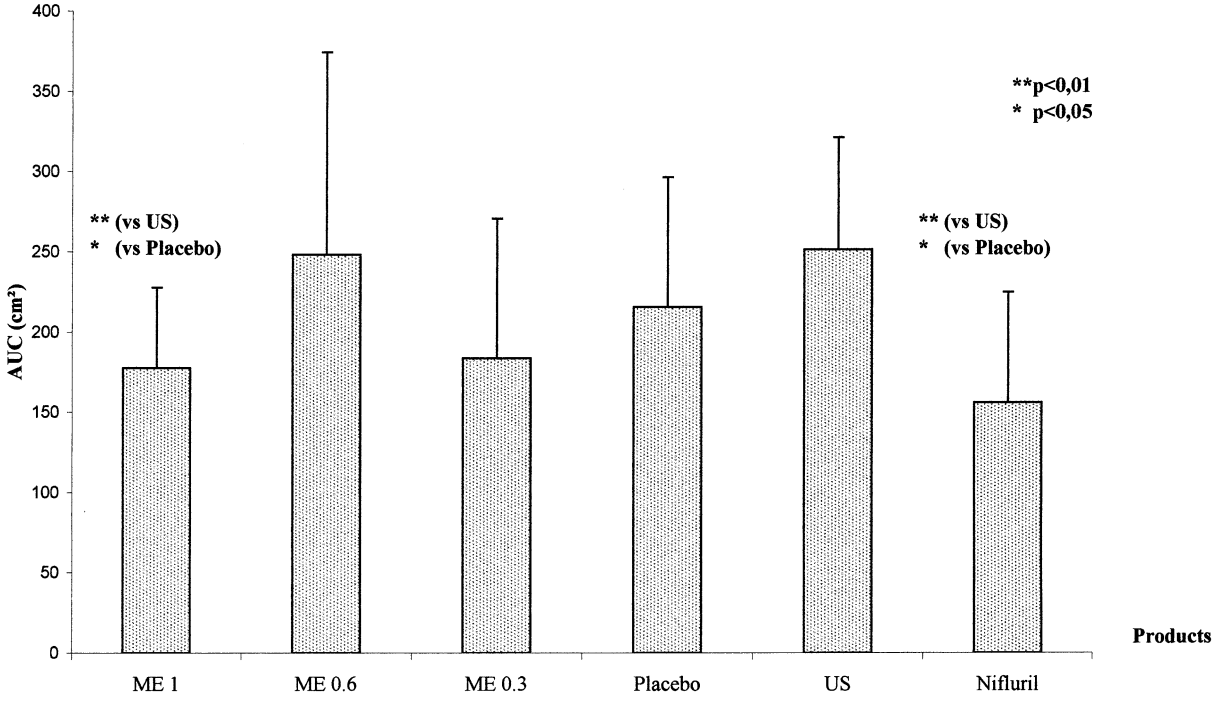

Fig. 3. AUC mean values (cm^2) of the four treated sites versus untreated site and placebo site ($n = 12$).

No significant difference is observed between the US AUC and the placebo ME AUC (respectively 251.4 ± 70 and 215 ± 81 cm^2; $p = 0.1$). The placebo ME exhibits no anti-inflammatory effect. The AUC value calculated for the Nifluril® ointment is lower at 156 ± 70 cm^2 and significantly different from the US AUC and the placebo ME values ($p < 0.01$). No statistical difference is observed between the AUC values of the ME 0.3 ($p = 0.3$) and ME 0.6 ($p = 0.4$) versus placebo ME. ME 0.3 and ME 0.6 do not elicit any anti-inflammatory response to the MN stimulus.

The AUC at the ME 1 treated site (177 ± 50 cm^2) reveals the efficacy of the preparation to induce an anti-inflammatory response versus placebo ($p < 0.05$).

(2) The percentage of inhibition of the vasodilation constitutes a useful parameter for the evaluation of the pharmacological activity of the preparation (Poelman et al., 1991) (Table 2). The inhibition percentages were respectively, 18% for ME 1 and 27% for Nifluril®.

(3) The average perfusion response curves versus time are shown in Fig. 4.

On the untreated skin the cutaneous vascular response to MN appears after 3–5 min. The start of the MN induced vasodilation is characterized by an increase in the slope of the curve. After this short period, the perfusion level reaches a plateau, followed by a progressive decrease. On the treated sites, the vasodilation behaviour, characterized by the perfusion level versus time curve, is quite different. Once erythema appeared, and increased, the level of redness is maintained for 15 min before gradually fading.

On the Nifluril® treated area, the perfusion values are lower and statistically different from

Table 2
Inhibition percentages of the vascular response on the treated sites

ME 0.3	ME 0.6	ME 1	Nifluril®
NS	NS	18	27

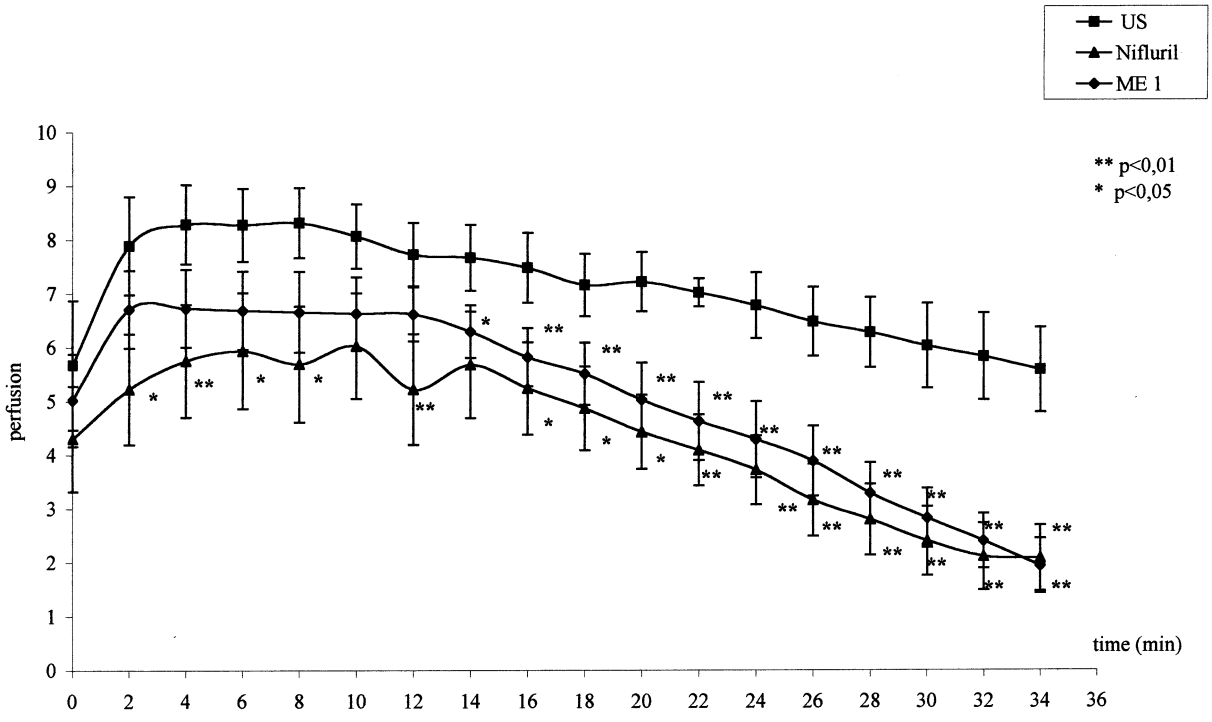

Fig. 4. Kinetics of the cutaneous blood flow on the treated sites (ME 1 and Nifluril) versus the untreated site.

the US values and the duration of the plateau is shortened. After 16 min the perfusion dramatically decreases.

On the ME 1 treated site, the perfusion values obtained at the plateau are not statistically different from the values obtained during the same period on the US site. The duration of the plateau is short (14 min). Then, the decrease of the perfusion is fast, and corresponds to the release of the drug.

(4) A decrease of the perfusion on the three perfusion profiles versus time was observed (Fig. 4). However, the mean slopes (s) calculated on the two treated sites curves are of the same order of magnitude ($s = 0.35$) and significantly different of the slope value for the untreated skin ($s = 0.1$) ($p < 0.01$).

4. Discussion

Until recently, pharmaceutical application of sucrose esters had remained sparsely reported (Brooks, 1980; Desai and Lowicki, 1985; Desai, 1990; Ntawukuliyayo et al., 1993; Thevenin et al., 1996).

This paper documents sucrose ester based microemulsion as drug carrier systems to deliver niflumic acid into the skin. High levels of surfactants could promote drug absorption by disrupting the closely packed skin barrier (Friberg, 1990; Ashton et al., 1992a and b, Rieger, 1995), but since sucrose esters are known to have excellent skin compatibility (Brooks, 1980), the effect of the present microemulsions could not be easily attributed to their surfactant content.

Microemulsions exhibit various phase equilibria in addition to rich structural diversity (Schubert and Kaler, 1996). It is obvious that their specific microstructure greatly influences their properties as a drug carrier system.

In this study, the FFEM technique, which is an effective probe to reveal the ME morphological diversity, confirmed the microstructure of the microemulsion.

Carlfors et al. (1991) have pointed out the role of bicontinuous systems, which may enhance the transdermal transport of lidocaine through the skin. Large amounts of water and oil can be solubilised by addition of small amounts of surfactants, increasing the drug loading in these systems. The highly dynamic character of bicontinuous phases may enhance the transdermal process due to wetting properties of the surfactant system. Mavon et al. (1997) have demonstrated the role of the skin wettability in percutaneous absorption, which is a diffusional phenomenon and, accordingly, is dependent on the contact area between the permeant and the skin surface. Thus, the very low interfacial tension of bicontinuous structures of the order of 10^{-2} mN/m could improve the wetting of the stratum corneum, which may facilitate the drug transfer.

Surfactants may interact with the lipids of the stratum corneum, reducing the barrier function by fluidising the intercellular lipids (Walters et al., 1982). Non ionic surfactants are known to cause little damages to the skin (Ashton et al., 1992a,b). According to Nobile et al. (1964) sucrose esters do not remove the cutaneous fat film and would not denature the protein of the skin surface in contrast to the usual ethoxylated non ionic surfactants.

Thus, it could be assumed that in the present ME system the role of the microstructure is far more important to enhance percutaneous absorption.

As the tested formulations are applied after MN, the experiments enable us to specifically measure their curative potential against inflammation. For the Nifluril® ointment, we found that under our conditions, the drug is immediately available to elicit a measurable pharmacological effect. Poelman et al. (1989) and Treffel and Gabard (1993) have reported that the MN skin inflammation assay was convenient to measure the bioavailability of NAIDS.

Treffel and Gabard (1993) have demonstrated that increasing amount of Ibuprofen in the epidermis resulted in greater inhibition of the vascular response of the MN assay.

We measured the percutaneous bioavailability of three increasing doses of niflumic acid (0.3, 0.6 and 1%) in the microemulsion vehicle.

The drug concentrations were respectively ten, five and three times lower than the concentration of niflumic acid in the commercially available o/w emulsion.

It should be noted that the inter-individual variations in the vascular responses to such low concentrations are higher than for the 1% niflumic acid microemulsion. This phenomenon could explain the lack of significance versus placebo of ME 0.3 and ME 0.6.

The lower AUC value associated to a smaller S.D. observed with 1% niflumic acid in the microemulsion suggests the better availability of the NSAID in this vehicle.

The ME 1 is saturated with niflumic acid and according to Higuchi (1960), the highest thermodynamic activity is then reached. The drug delivery to the target site is favoured (Kemken et al., 1992).

However, in the ME 1, the anti inflammatory activity occurs only after a lag period of 16 min.

This phenomenon is related to the weak solubility of niflumic acid in the oily and water phases. Then, the drug is mainly concentrated in the interfacial film, which could control the release of the NSAID. As previously demonstrated by Meloni et al. (1994) and Gasco et al. (1990), the microemulsion could act as a drug reservoir. Gasco et al. have studied the release of doxorubicin, a hydrophilic molecule, from o/w and w/o microemulsions. No diffusion of the drug from the o/w microemulsion was noted. They explained this fact by the interaction of the drug with the surfactant molecules. They concluded that the formation of complexes between the surfactants and a drug with dissociable groups could greatly change its effective solubility and lipophilicity. The variation of lipophilicity of a drug, due to the interactions with surfactants can therefore modify the reservoir effect of the disperse phase of microemulsions.

The difference observed between the o/w emulsion and the active ME, in terms of the percentage of inhibition of the drug is explained by the progressive release of niflumic acid from the ME vehicle which delayed the onset of the anti-inflammatory process.

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