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ScienceDirect
International Journal of Pharmaceutics 346 (2008) 119–123
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Positively charged microemulsions for topical application

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Received 19 January 2007; received in revised form 25 May 2007; accepted 26 May 2007
Available online 3 June 2007

Abstract

The study reports pig-skin permeation and skin accumulation of miconazole nitrate (MCZ) from positively charged microemulsions containing water, 1-decanol/1-dodecanol (2:1, w/w), lecithin and/or decyl polyglucoside at different weight ratios, propylene glycol, 1,2 hexanediol and a cationic charge-inducing agent (stearylamine (ST), L-alanine benzyl ester (ALAB) or cetyltrimethylammonium bromide (CTAB)). Zeta-potential values of the positively charged microemulsions ranged from 14.2 to 37.5 mV and mean droplet size from 6.0 to 16.8 nm.

In vitro pig-skin permeation of MCZ after a single 24 h application was negligible for all microemulsions; accumulation from positively charged microemulsions was nearly twice that from their negatively charged counterparts.

The increased accumulation might be ascribed to the interaction between positive microemulsive systems and negatively charged skin sites; no significant difference was observed among the various cationic charge-inducing agents.

Skin accumulation from the microemulsion containing most lecithin was lower than those of other microemulsions; this was ascribed to the phase transformation from microemulsion to a liquid crystal system after skin contact.

These results suggest that positively charged microemulsions could be used to optimize drug targeting without a concomitant increase in systemic absorption; ALAB, an ester of a natural aminoacid, is an appropriate cationic charge-inducing agent.

Keywords: Positively charged microemulsion; Miconazole; Topical application; Skin accumulation

1. Introduction

Epithelial cells carry a negative charge upon their surface, due to the presence of negatively charged protein residues on the outer side of their membranes, and to selective active ion pumps (Rojanasakul et al., 1992). All epithelia should thus be selective to positively charged delivery systems that interact with cells, leading to increased drug permeability and prolonging the pharmacological effect.

In recent years, positively charged liposomes and emulsions have been used as drug carriers. Positively charged liposomes prepared with stearylamine (ST), a cationic lipid, have been used for ocular delivery of Acyclovir (ACV) (Law et al., 2000). Since the cornea provides a negatively charged surface it allows positively charged liposomes to permeate it. Positively charged liposomes increased absorption of ACV, because of a stronger binding effect to the cornea surface than occurs with negatively charged liposomes.

It has also been shown (Nagarsenker et al., 1999) that incorporation of ST into liposomes results in more consistent and higher AUC values for tropicamide; this formulation was found to be more effective in dilating the pupil than drug-loaded neutral liposomes.

Elbaz et al. (1993) prepared the first positively charged submicron emulsion using stearylamine. It was later demonstrated that a positively charged submicron emulsion formulation of piroxicam used in the management of corneal alkali-burning had a pronounced effect on both ulceration rate and epithelial defects compared to all the other test treatments, such as piroxicam solution or blank emulsion (Klang et al., 1999).

Drug-loaded positively charged submicron emulsions might also bind to negatively charged sites present on the skin, making the development of topically applied positively charged submicron emulsion promising. This hypothesis is supported by the results of Conrads and Zahn (1987) who showed maximum binding of anionic surfactants with the stratum corneum at pH

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doi:10.1016/j.ijpharm.2007.05.065

2, and decreased binding with increased pH. It can therefore be deduced that the stratum corneum is a positively charged membrane at low pH, and becomes negatively charged at high pH, above the isoelectric point of keratin.

Recently, positively charged submicron emulsions of about 130 nm, incorporating econazole and miconazole nitrate, have been proposed as a new type of colloidal drug carrier (Youenang Piemi et al., 1999).

During the last decade, numerous studies have suggested that a novel type of vehicle, i.e. the microemulsion, has the potential to increase cutaneous drug delivery of both hydrophilic and lipophilic drugs compared to conventional vehicles, and thereby fulfil the many promises of the cutaneous drug-delivery route (Kreilgaard, 2002).

Microemulsions are a special class of transparent dispersions, which actually have little in common with emulsions (Tadros et al., 2004). Unlike submicron emulsions, which are only kinetically stable, microemulsions are thermodynamically stable systems and are characterized by a highly-dispersed internal phase with droplet size ranging from 5 to 50 nm. As a consequence of their thermodynamic stability, microemulsions can be prepared by an inexpensive process through autoemulsification. These dispersed systems are isotropic, as opposed to liquid crystals which are anisotropic (Langevin et al., 1985). Moreover, investigations have shown that the unique structural organization of the phases in microemulsions may contribute to additional solubility regions, increasing their loading capacity versus non-structured solutions containing the same fractions of the constituents (Kreilgaard, 2002). As far as the authors have been able to determine, no reports have described positively charged microemulsions for pharmaceutical applications, so it is of interest to verify the potential of such systems.

MCZ, one of the broad-spectrum antifungal agents, is a weak base ($pK_a = 6.7$) characterized by relatively high molecular weight and melting point. This drug is poorly soluble in water (1.03 μg/ml) (Pedersen et al., 1993) and in mineral oil (10 μg/ml) (Fujii et al., 2002), which reduces its efficacy for many therapeutic applications. MCZ is usually employed at 2% (w/w) in topical suspensions for the treatment of dermatophytoses, superficial mycoses and mixed infections, or as an oral gel in treating Candidal infections; two to daily applications are required.

The aim of the present research was to develop and characterize positively charged microemulsions containing MCZ and to investigate the in vitro behavior of the formulations, using pig-skin.

2. Materials and methods

2.1. Materials

Soybean lecithin (Epikuron® 200, phosphatidylcholine content above 95%) was from Lucas Meyer (Hamburg, G) and was used without further purification. 1-Decanol, 1-dodecanol, and 1,2-hexanediol were from Fluka (Buchs, CH); decyl polyglucoside (Oramix® NS 10) was from Seppic (Milan, I). Cetyltrimethylammonium bromide (CTAB) was from Merck (Darmstadt, D). Propylene glycol (PG) was from Carlo Erba (Rodano, Milan, I), miconazole nitrate (MCZ), stearylamine (ST), and L-alanine benzyl ester (ALAB) were from Sigma (St. Louis, MO, USA). Distilled water was purified using a Milli-Q system (Millipore®, Bedford, MO). All chemicals were of analytical grade.

Table 1
Microemulsion compositions

Components	M1 (%, w/w)	M2 (%, w/w)	M3 (%, w/w)
1-Decanol/1-dodecanol (2:1)	1.61	1.59	1.53
Epikuron 200	6.22	1.90	–
Oramix NS 10	4.35	12.00	13.04
1,2-Hexanediol	6.80	4.20	3.38
Propylene glycol	11.60	9.80	15.95
Phosphate buffer pH 5.8	68.42	69.51	65.10
Miconazole nitrate	1.00	1.00	1.00

2.2. Microemulsion formulations

Microemulsions were prepared by weight using appropriate amounts of oil phase (1-decanol:1-dodecanol 2:1, w/w), surfactant (Oramix® NS 10 or a mixture of lecithin and Oramix® NS 10), aqueous phase (phosphate buffer 0.01 M at pH 5.8) and co-surfactant (PG and 1,2-hexanediol). The microemulsion compositions (w/w) are given in Table 1. Drug-loaded microemulsions were obtained by dissolving MCZ (1.0%, w/w) in the previously prepared microemulsion.

One of three different charge-inducing agents was added to the various batches of drug-loaded microemulsions: ST (1%, w/w), ALAB (2%, w/w) or CTAB (1.2%, w/w).

2.3. Physical characterization

Microemulsion droplet size was determined at 25 °C by photon correlation spectroscopy (PCS) using light-scattering equipment (90 Plus, Brookhaven Instruments Corporation, New York, USA). Measurements were obtained at an angle of 90° on microemulsions after filtration through a microporous filter with 0.45 μm pore diameter (Millipore®). Scattering intensity data were analyzed with a digital correlator and fitted by the inverse Laplace transformation method. Each system was analyzed twice, and ten size determinations were made for each sample. Viscosity values were determined in triplicate using an Ubbelohde micro-viscometer (Schott-Geräte, Hofheim, D).

Zeta potential of the microemulsions was measured using the ZetaPlus-Zeta Potential Analyzer (Brookhaven Instruments Corporation, New York, USA). Measurements were obtained on microemulsions after filtration through a microporous filter with 0.45 μm pore diameter. Each system was analyzed twice, and ten size determinations were made for each sample.

After 6 h application onto the skin the microemulsions were recovered and observed under polarized light to distinguish between microemulsion systems (isotropic) and liquid crystals (anisotropic).

2.4. In vitro permeation and skin deposition studies

Full-thickness pig ear skin was used for permeation experiments using a vertical cell, as proposed by Franz (Franz, 1975). Excised skin was rinsed with normal saline and pre-hydrated by floating it with the stratum corneum upward on 0.002% (w/v) aqueous sodium azide to maintain an in vivo transepidermal hydratation. The skin was then sandwiched between ground glass with the stratum corneum side upwards. The receptor chamber was filled with 6 ml of phosphate buffer solution at pH 7.4 containing 20% (w/w) propylene glycol. Drug solubility in the receiving medium determined at 37 °C was 1.3 mg/ml. The test formulations (500 μl) were applied to the skin surface, which had an available diffusion area of 1.7 cm^2. The content of the receptor cell, continuously stirred at 300 rpm by a rod-shaped magnet and thermostated at 37 °C, was removed at appropriate intervals for HPLC determination, and the cell was immediately refilled with fresh receptor solution.

After 24 h, the skin surface was washed five times with ethanol:water (1:1) followed by water, to remove excess drug from the surface. The skin was then cut into small pieces, homogenized with methanol and left for 6 h at room temperature. After shaking for 5 min, it was centrifuged for 5 min at 5000 rpm and the supernatant analyzed by HPLC for MCZ determination. Each experiment was repeated at least in triplicate from two different batches of formulation.

Some experiments were carried out after pre-treating the skin for 6 h with drug-free microemulsion containing ALAB, and then washing it five times with ethanol:water (1:1) followed by water. It was then used for MCZ permeation studies from ALAB-free microemulsion.

2.5. MCZ analysis

MCZ content was determined by using an HPLC apparatus consisting of a pump (LC 10-AD), a UV detector (SPD-10A) and a data station (Shimadzu, Kyoto, Japan). An Ultrasphere® C-18 column (250 mm × 4.6 mm i.d., 5 μm particle size, Waters, Milford, MA) was used. After mixing, the mobile phase, which comprised methanol:phosphate buffer solution at pH 3.0 (90:10, v/v), was degassed. The eluent was run at a rate of 1.0 ml/min and monitored at 230 nm following injected volumes of 20 μl. The relative retention time was found to be 7.2 min. A calibration graph was constructed in the 5–100 μmol/l range (eight points, each determined in quadruplicate); its linearity was shown by the R^2 coefficient value (0.9985) of the regression equation:

$$y = 2 \times 10^{10}x + 34458$$

The LOQ, defined as the lowest MCZ concentration in the curve that can be measured routinely with acceptable precision and accuracy, was 10.0 μmol/l; the LOD, defined as the lower detection limit, was 2.0 μmol/l (signal to noise > 2.0).

2.6. Data report

Each set of skin uptake experiments was performed at least three times. Results are reported as means ± standard deviations (S.D.); statistical analysis was via a two-tailed Student's test.

3. Results and discussion

Microemulsion components were chosen taking their biocompatibility into account; in particular, lecithin and decyl polyglucoside (Oramix® NS 10) were used as surfactants. Natural lecithins are normal constituents of biological membranes and have no toxic effects even at high concentrations. Alkyl polyglucosides are a mixture of glucosides from degraded starch fractions; they are stable at high pH values, but sensitive to low pH values, at which they hydrolyze to glucose and fatty alcohol. The main attraction of these sugar-based surfactants is their favorable environmental profile, as the rate of biodegradation is usually high while toxicity is low. In addition, alkyl polyglucosides have favorable dermatological properties, being very mild to the skin and eye (Manconi et al., 2006). A mixture of 1-decanol:1-dodecanol 2:1 (w/w), in which drug-solubility was determined to be 0.44 mg/ml, was used as oil phase.

To obtain microemulsion systems at low surfactant concentration, co-solvents can be added (Kreilgaard, 2002); for this purpose we used propylene glycol and 1,2 hexanediol, pharmaceutically acceptable ingredients.

The antifungal activity of the imidazole derivatives, miconazole and ketoconazole, is reported to be reduced when they were entrapped in liposomal structures. The association of phospholipids with imidazole inhibits the antifungal activity, similarly to the effect observed when miconazole or ketoconazole are entrapped in SUV liposomes (De Logu et al., 2000). Accordingly, to avoid similar effects we progressively decreased the amount of lecithin used as surfactant in the microemulsion systems. The microemulsion M3 only contained decyl polyglucoside as surfactant (Table 1).

As a consequence of the supersolvent properties of microemulsions, MCZ was solubilized at a relatively high concentration (1.0%, w/w).

Cationic charge-inducing agents were added to the microemulsions. Particularly, ST and CTAB were employed only for comparison purposes, as their toxic effects would need to be taken into account for practical use; ALAB, an ester of a basic aminoacid with low toxicity (Gorecki et al., 1980), was used as a suitable alternative molecule.

The zeta potential of all cationic microemulsions was positive (Table 2). The positive surface potential value of the microemulsion droplets was probably due to the presence of the cationic agent in its ionized form at the oil/water interface. Moreover, the introduction of cationic charge-inducing agents in the formulation determined no significant variation in viscosity or mean diameter compared to microemulsions without cationic agents.

Skin permeation experiments (Table 3) revealed that undetectable amounts (<0.8 μg/ml) of drug were found in the Franz-cell receptor solution. Skin accumulation values of MCZ from microemulsion M1 were lower than from M2 and M3, although the presence of cationic agents in microemulsions increased accumulation about twofold compared to their negative counterparts. Analysis in polarized light may explain these low accumulation values: Fig. 1 shows that, after 6 h contact with pig-skin, system M1 was birefringent, unlike systems M2 and M3. This was attributed to a variation in the microemulsion water

Table 2
Viscosity, mean diameter, and zeta potential of the microemulsions

Microemulsion	Viscosity ± S.D. (mPa s)	Mean diameter ± S.D. (nm)	Zeta potential ± S.D. (mV)
M1	11.6 ± 0.4	6.5 ± 1.5	−30.4 ± 0.1
M1 ST	11.7 ± 0.3	7.3 ± 2.3	27.0 ± 3.8
M1 ALAB	11.6 ± 0.5	6.2 ± 2.8	32.6 ± 4.3
M1 CTAB	11.8 ± 0.4	6.0 ± 0.5	28.0 ± 2.5
M2	6.5 ± 0.2	16.8 ± 4.6	−30.8 ± 0.1
M2 ST	6.7 ± 0.2	13.2 ± 5.1	37.5 ± 4.0
M2 ALAB	6.4 ± 0.3	6.8 ± 1.2	35.7 ± 2.9
M2 CTAB	6.6 ± 0.3	9.1 ± 3.8	30.2 ± 1.5
M3	6.1 ± 0.3	7.0 ± 2.3	−33.2 ± 0.6
M3 ST	6.6 ± 0.3	10.8 ± 3.8	15.0 ± 1.9
M3 ALAB	6.2 ± 0.1	6.7 ± 1.3	14.2 ± 2.1
M3 CTAB	6.0 ± 0.4	6.3 ± 1.8	17.4 ± 2.5

Table 3
Skin permeation and skin accumulation of miconazole

Microemulsion	Flux ($\mu g\,cm^{-2}\,h^{-1}$)	Skin accumulation ± S.D. ($\mu g/cm^2$)
M1	Negligible	24 ± 3
M1 ST	Negligible	53 ± 6
M1 ALAB	Negligible	52 ± 5
M1 CTAB	Negligible	59 ± 7
M2	Negligible	68 ± 7
M2 ST	Negligible	153 ± 22
M2 ALAB	Negligible	122 ± 15
M2 CTAB	Negligible	102 ± 12
M3	Negligible	73 ± 8
M3 ST	Negligible	164 ± 15
M3 ALAB	Negligible	175 ± 20
M3 CTAB	Negligible	173 ± 17

Negligible <1.5 $\mu g\,cm^{-2}\,h^{-1}$.

content producing a phase transformation from microemulsion to a liquid-crystal system. This transformation decreased drug release, as is also reported in the literature (Mueller-Goymann and Hamann, 1993; Trotta, 1999); reduction of drug diffusion coefficients by a factor as high as 100 has been reported.

MCZ skin accumulation from positively charged microemulsions was nearly double that from negatively charged ones. In particular, to determine whether the enhanced skin-transport of MCZ is attributable to direct damage of the skin by the aminoacid ester, permeation experiments were carried out after pre-treating the skin with drug-free microemulsion containing ALAB. After skin pre-treatment, MCZ fluxes from the negatively charged microemulsions were undetectable and skin accumulation did not change significantly (75 ± 7 $\mu g/cm^2$) compared to accumulation from the same microemulsions without pre-treatment.

When lecithin was removed from the microemulsions, MCZ skin accumulation further increased, as can be seen from the values obtained for M3, which were higher than those for M2.

Youenang Piemi et al., 1999 reported that a positively charged submicron emulsion promoted the diffusion rate of MCZ through hairless female rat skin, with skin accumulation of about 16 $\mu g/cm^2$. MCZ accumulation in snakeskin after 24 h local application of an oil suspension and a water suspension was 1.4 and 31 $\mu g/cm^2$, respectively (Fujii et al., 2002), and human skin accumulation of about 5 $\mu g/cm^2$ after 16.6 h application of 1% cream base has also been reported (Shaeffer and Stüttgen, 1976). These reported values are much lower than those found for the microemulsions studied here, in which, after 24 h topical application, skin accumulation was about 70 $\mu g/cm^2$ from negatively charged microemulsions and reached a value of 170 $\mu g/cm^2$ from the positively charged microemulsions.

The favorable drug-delivery properties of microemulsions are attributable to the concentration gradients provided by the high drug-solubility potential of the vehicles. Since the object of topical MCZ formulations is to deliver the drug locally to the skin, it is interesting to note that remarkable skin accumula-

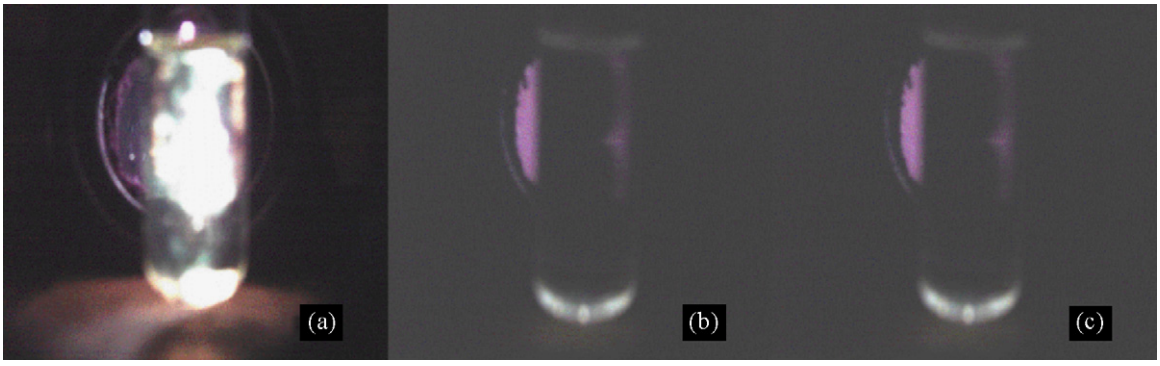

Fig. 1. Polarised light photography of the microemulsions after 6 h application (a) M1, (b) M2 and (c) M3.

tion with negligible drug flux optimizes drug targeting without a concomitant increase in systemic side effects.

4. Conclusions

This study suggests the effectiveness of positively and negatively charged microemulsions in promoting drug accumulation in pig-skin. Microemulsions containing MCZ were formulated with components considered safe for topical application, and ALAB, a molecule with low toxicity, was employed as cationic inducing-agent for the positively charged microemulsion formulations. Skin accumulation of MCZ from the positively charged microemulsions was higher than that from their negatively charged counterparts, and was attributed to the binding affinity of the droplets for the skin. Positively charged microemulsions appear to be a promising formulation for topical drug delivery, confirming the potential use of these vehicles.

Acknowledgements

This study was supported by a grant from Ministero dell'Istruzione, dell'Università e della Ricerca.

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