Skin-compatible lecithin drug delivery systems for fluconazole: effect of phosphatidylethanolamine and oleic acid on skin permeation

Sonja Hoeller, Victoria Klang and Claudia Valenta

Abstract

The purpose of the present study was to evaluate skin-compatible drug delivery systems for fluconazole. Pseudoternary phase diagrams were constructed, composed of different soybean lecithins/oil/isopropanol and water. The role of the various lecithin compositions was expressed in the different resulting isotropic areas. Based on these phase diagrams, two systems were chosen as drug delivery systems for fluconazole. The influence of phosphatidylethanolamine and of the oil component on the skin permeation of fluconazole was investigated. The more phosphatidylethanolamine, the greater was the fluconazole skin permeation, independent of the hydrophilicity of the system. The influence of oleic acid and isopropylmyristate as the oil component was compared and a greater penetration enhancing effect was found for the microemulsion containing oleic acid.

Introduction

Microemulsions are clear isotropic, thermodynamically stable liquids with ultralow interfacial tension, large interfacial area and the capacity to solubilize both aqueous and oil-soluble compounds (Paul & Moulik 2001). The different resulting structures that were formed by mixing various ratios of surfactants have been studied and systematized in detail (Aboofazeli & Lawrence 1993, 1994, 1995; Wang et al 2006). These self-assembled structures are tuneable by changing the ratio and the concentrations of the ingredients.

In order to affect flux through the skin, several permeation enhancers have been used in such formulations. Oleic acid (OA) and several other fatty acids are able to interrupt the lipid barrier in the stratum corneum by forming separate domains that interfere with the continuity of the multilamellar stratum corneum and may induce highly permeable pathways (Ongpipattanakul et al 1991; Hadgraft 2001; Peltola et al 2003). Also, isopropylmyristate (IPM), another non-toxic ingredient, is well known as a permeation enhancer in transdermal formulations, although the mechanism of its action is poorly understood (Goldberg-Cettina et al 1995).

The aim of the present study was to construct pseudoternary phase diagrams, consisting of lecithin/isopropanol/oil and water, in order to determine how different commercially available soybean lecithins influence the formation of microemulsions and transport of the entrapped lipophilic drug through the skin. The influence of OA or IPM as the oil component was also compared.

Material and Methods

Materials

Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Life Sciences, Althanstrasse 14, 1090 Vienna, Austria

Sonja Hoeller, Victoria Klang, Claudia Valenta

Correspondence: C. Valenta, Department of Pharmaceutical Technology and Biopharmaceutics, Faculty of Life Sciences, Althanstrasse 14, 1090 Vienna, Austria. E-mail: claudia.valenta@univie.ac.at

Fluconazole (CAS: 86386-73-4) was purchased from Kemprotec (Middlesbrough, UK). Soybean lecithins with different concentrations of phosphatidylcholine (PC), lysophosphatidylcholine (LC) and phosphatidylethanolamine (PE), namely Lipoid S45 (CAS: 745303-1), Lipoid S75 (CAS: 776099-1) and Lipoid S100 (CAS: 790535-5) were kindly donated by Lipoid GmbH (Ludwigshafen, Germany). Isopropanol was supplied by Merck (Hohenbrunn, Germany). IPM was purchased from Sigma-Aldrich (St Louis, MO, USA)

and OA (EG.Nr: 204-007-1) was supplied by Herba Chemosan (Vienna, Austria).

Construction of pseudoternary phase diagrams

In order to construct pseudoternary phase diagrams, appropriate amounts of three different lecithins (S100, S75 and S45), OA and isopropanol were weighed into vials and stirred at room temperature until a clear solution was obtained. Distilled water was added drop by drop and stirred to attain equilibrium. After equilibration, the mixture was assessed visually and under cross polarizers for the absence of a liquid crystalline phase in order to determine the boundaries of microemulsions identified by isotropic phases and birefringent liquid crystalline domains.

Microscopic characterization of microemulsions

Polarizing light microscopy studies were carried out at room temperature using an Optiphot microscope (Nikon GmbH, Germany) in order to get more information on the microscopic structure of the systems.

Formulations

The formulations were prepared as described and the compositions are presented in Table 1. System 1 consisted of 25% oil, 25% surfactant, 25% co-surfactant and 25% water. System II consisted of 40% oil, 25% surfactant, 25% co-surfactant and 10% water. Both systems were also prepared with OA and IPM.

In-vitro skin permeation study

Porcine abdominal skin was shaved and then prepared with a dermatome (GB 228R; Aesculap, Tuttlingen, Germany) set at 1.0 mm. The permeation of fluconazole was investigated as described previously (Hoeller & Valenta 2007).

High-performance liquid chromatography

Fluconazole was quantified by high-performance liquid chromatography (Perkin Elmer, USA) using a previously reported procedure (Hoeller & Valenta 2007). Calibrations curves were calculated on the basis of peak area measurements of standard solutions. The correlation coefficient was 0.9993. The concentration range of the standard solution for fluconazole was between 13.66 and 109.3 μg mL^{-1}.

Statistical analysis

Results are expressed as the means of at least three experiments$\pm$s.d. Statistical analysis for Figure 3 was performed using the Kruskal–Wallis test ($P < 0.05$).

Results

Microscopic characterization of microemulsions

The microscopic characterization of the microemulsions is presented in Figure 1. Microemulsions were identified by their clear isotropic texture, which appears under polarized light as black planes (Figure 1A). Such phases are usually produced at very high surfactant concentrations and low water content. As the surfactant concentration was further diluted with water, fluent drop aggregates formed, as shown in Figure 1B. Typical 'mosaic' textures (Figure 1C) appeared mostly at a very low surfactant concentration of about 10–20%. At a higher surfactant concentration of about 25–30%, various liquid crystalline structures were obtained (Figure 1D–F). Such structures showed birefringence under polarized light and appeared mostly at a mixing ratio with low oil content (10–20%). At a surfactant/co-surfactant concentration of about 30%, typically lamellar liquid crystalline structures were obtained, while at higher surfactant concentrations, hexagonal phases were produced.

Table 1 Composition of the microemulsions (% w/w)

Formulation	Isopropylmyristate	Oleic acid	Lipoid S100	Lipoid S 75	Lipoid S45	Isopropanol	Water	Fluconazole
System I								
I-1	25	–	25	–	–	25	25	1
I-2	25	–	–	25	–	25	25	1
I-3	25	–	–	–	25	25	25	1
I-4	–	25	25	–	–	25	25	1
I-5	–	25	–	25	–	25	25	1
I-6	–	25	–	–	25	25	25	1
System II								
II-1	40	–	25	–	–	25	10	1
II-2	40	–	–	25	–	25	10	1
II-3	40	–	–	–	25	25	10	1
II-4	–	40	25	–	–	25	10	1
II-5	–	40	–	25	–	25	10	1
II-6	–	40	–	–	25	25	10	1

Lipoid S100: 98.40% phosphatidylcholine (PC), 0.3% lysophosphatidylcholine (LC) and < 0.1% phosphatidylethanolamine (PE); Lipoid S75: 69.90% PC, 2.20% LC and 8.40% PE; Lipoid S45: 54.90% PC, 1.0% LC and 16.90% PE.
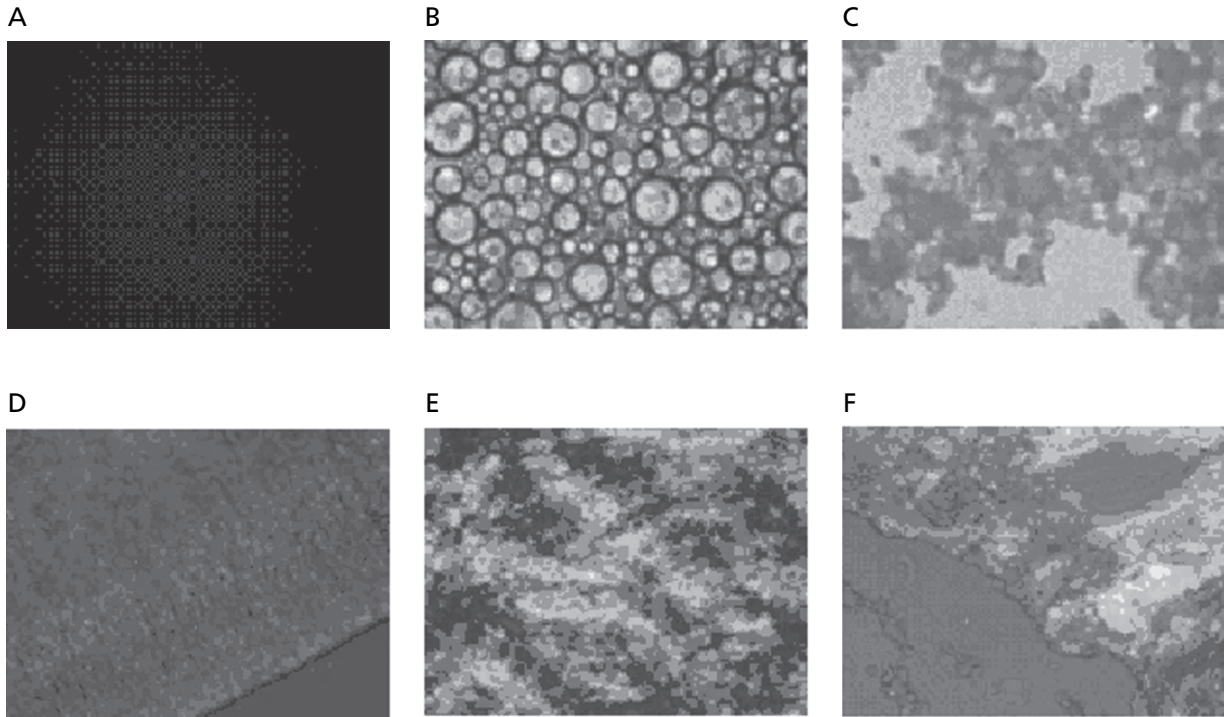
Figure 1   Micrographs of the phase textures obtained by the construction of the pseudoternary phase diagrams. Surfactant–cosurfactant/oil/water: A. isotropic texture: 50/40/10; B. fluent drop aggregates: 10/10/80; C. mosaic texture: 20/30/50; D–F. liquid crystalline textures: 30/10/60; 30/10/60; 30/10/60.

Pseudoternary phase diagrams

The overlaid pseudoternary phase diagrams are presented in Figure 2. The largest isotropic area was obtained by Lipoid S75, which contains 69.90% of phosphatidylcholine, followed by the narrowed area induced by Lipoid S100, with the highest content of phosphatidylcholine. A comparison of the isotropic area from S45 (lowest amount of PC) with S75 and S100 showed an extension in the water and oil region. The possible compositions for isotropic regions are approximately 45–85% of surfactant/co-surfactant, 5–55% of oil and 5–30% of water.

In-vitro skin permeation study

The results are presented in Figure 3 and the cumulative amount of the permeation of fluconazole is included in Table 2.

System I

The microemulsion with OA showed a greater cumulative amount of fluconazole permeated after 48 h of diffusion through the skin than those with IPM (Figure 3A). The rank order of the cumulative amount permeated in the case of OA was: I-6 > I-5 > I-4. The greatest permeation was obtained from I-6. This was the formulation with the lowest content of PC (S45), but a high content of PE (19.90%). In second position was the lecithin (S75) with 8.4% PE. The formulation with the highest amount of PC, but almost no PE, showed lower skin permeation (S100). Regarding the formulation with IPM, the greatest permeability was obtained from I-2.
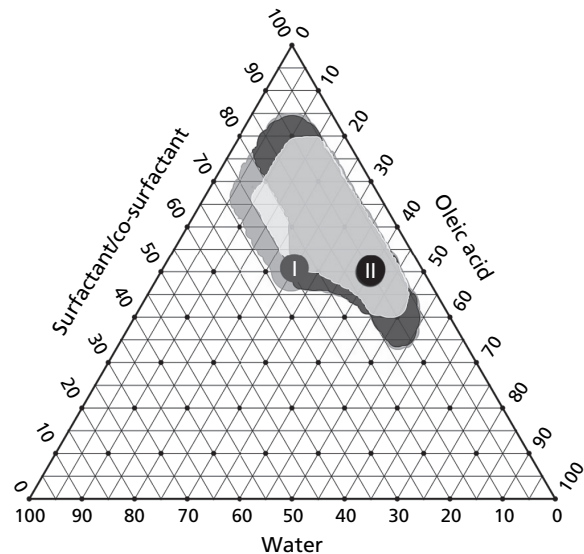

Figure 2   Overlaid pseudoternary phase diagrams of the oil–surfactant/co-surfactant–water systems using three different lipid compositions produced at room temperature (the marked area represents the microemulsion region); pale grey: Lipoid S75; black: Lipoid S100; white: Lipoid S45. Point I: System I consisting of 25% oil, 25% lipid, 25% isopropanol and 25% water. Point II: System II consisting of 40% oil, 25% lipid, 25% isopropanol and 10% water.
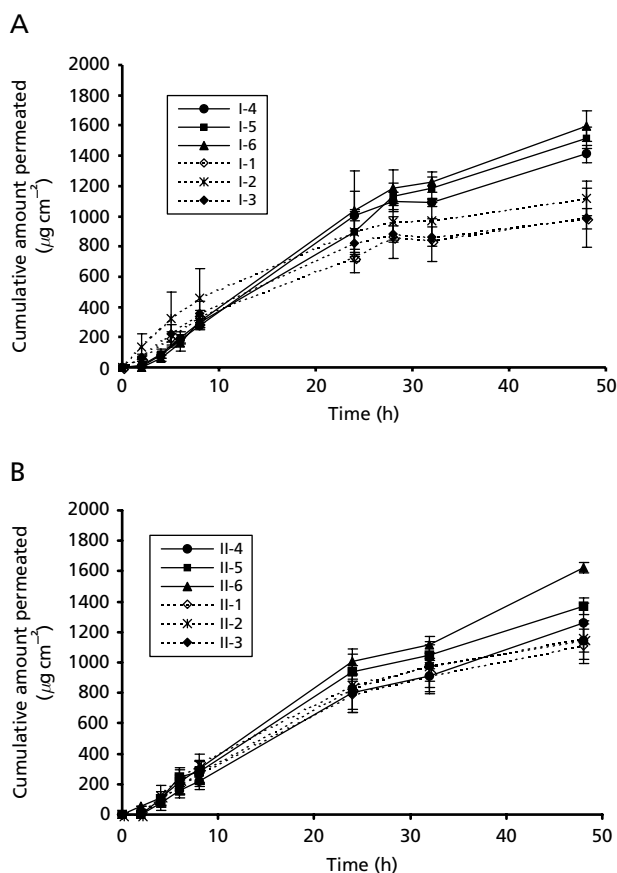
A

B

Figure 3   Fluconazole diffusion through excised porcine skin after 48 h from microemulsion. A. Microemulsion system I; B. microemulsion system II; n = 3.

Table 2   Comparison of the cumulative drug amount ($\mu g\ cm^{-2}$) of fluconazole permeated after 48 h of diffusion through porcine skin (n = 3)

Formulation code	Cumulated drug amount permeated ($\mu g\ cm^{-2}$)
System I	
I-1	988.99 ± 197.44
I-2	1119.97 ± 114.90
I-3	981.66 ± 68.51
I-4	1411.97 ± 55.34
I-5	1517.24 ± 71.35
I-6	1596.65 ± 102.89
System II	
II-1	1106.81 ± 114.90
II-2	1160.76 ± 93.89
II-3	1147.01 ± 124.56
II-4	1258.80 ± 133.87
II-5	1372.57 ± 54.48
II-6	1622.84 ± 31.32

Approximately the same amount of fluconazole was released from I-1 and I-3. A comparison of the permeation of I-1, I-2 and I-3 with I-6 containing OA, indicated a significantly greater cumulative amount of fluconazole permeated from the latter ($P < 0.05$, Kruskal-Wallis-Test).

System II

In System II, a greater cumulative amount of fluconazole permeated through the skin from the preparation containing OA (Figure 3B). Comparing II-3, which contained IPM, with II-6, which contained OA, indicated a significantly greater cumulative amount of fluconazole permeated from II-6 ($P < 0.05$, Kruskal–Wallis test). The rank order of the results regarding lecithin composition was (as in the case with OA): II-6 > II-5 > II-4, and in the case with IPM: II-2 > II-3 > II-1. Again, the microemulsion II-6, which contained the highest amount of PE, showed the greatest cumulative amount of fluconazole permeated through the skin, in contrast to II-4 and II-5. The cumulative amounts of fluconazole permeated after 48 h of diffusion are presented in Table 2.

Discussion

Lecithin has been shown to enhance skin penetration by interfering with skin lipids and therefore inducing a change in the skin lipid fluidity (Paolino et al 2002). The aim of the present study was to determine if the composition of different lecithins has an impact on the isotropic phase as well as on skin permeation. The phase diagrams show the different sizes of the isotropic area. Lipoid S75 achieved the biggest area. The components lysophosphatidylcholine and phosphatidylethanolamine may be responsible for this. Lipoid S45 also contains these ingredients and they may influence the effective critical packing parameter in a different manner as described by Aboofazeli & Lawrence (1994). In one study, the effect of PC and PE was investigated with respect to the transepidermal water loss: PC and PE at a concentration of $10\ mg\ cm^{-2}$ significantly reduced the transepidermal water loss, and PE was approximately 2-fold more effective than PC (Raney Sameersingh & Hope 2006). These data are consistent with the formation of extensive hydrophobic interactions between the skin and the outwardly facing acyl chains of the inverted hexagonal phase adapted by PE. In our case, the amount of PE applied was approximately $25\ mg\ cm^{-2}$, calculated from the applied formulation of 0.6 g containing 25% Lipoid S45 and containing approximately 17% PC. The applied doses would be sufficient to induce such hydrophobic interactions between skin and phosphatidylethanolamine. This could be one reason for the increase of fluconazole permeation.

OA and IPM were analysed for their percutaneous enhancing effect. The greatest permeation of fluconazole was achieved with OA as the oil component. This result seems compatible with the main mechanism suggested for the action of OA as a permeation enhancer, that is increasing stratum corneum permeability. Therefore, facilitated membrane diffusion could be achieved (Moser et al 2001; Touitou et al 2002). It has been shown that OA is more effective with highly hydrophilic compounds, which supports its use in combination with the rather hydrophilic fluconazole. In our study, this hypothesis was confirmed because in the more hydrophilic microemulsion I, the penetration enhancing effect of OA was more pronounced than in the more lipophilic microemulsion II.

In conclusion, it is possible to create skin-compatible lecithin microemulsions having high fluconazole diffusion

through porcine skin; the phosphatidylethanolamine content seems to play a major role in increasing the skin permeability.

References

Aboofazeli, R., Lawrence, M. J. (1993) Investigations into the formation and characterization of phospholipid microemulsions. I. Pseudo-ternary phase diagrams of systems containing water-lecithin-alcohol-isopropylmyristate. *Int. J. Pharm.* **93**: 161–175

Aboofazeli, R., Lawrence, M. J. (1994) Investigations into the formation and characterization of phospholipid microemulsions. II. Pseudo-ternary phase diagrams of systems containing water-lecithin-alcohol-isopropylmyristate and alcohol: influence of purity of lecithin. *Int. J. Pharm.* **106**: 51–61

Aboofazeli, R., Lawrence, M. J. (1995) Investigations into the formation and characterization of phospholipid microemulsions. IV. Pseudo-ternary phase diagrams of systems containing water-lecithin-alcohol and oil; the influence of oil. *Int. J. Pharm.* **125**: 107–116

Goldberg-Cettina, M., Liu, P., Nightingale, J., Kurihara-Bergstrom, T. (1995) Enhanced transdermal delivery of estradiol in vitro using binary vehicles of isopropylmyristate and short-chain alkanols. *Int. J. Pharm.* **114**: 237–245

Hadgraft, J. (2001) Skin, the final frontier. *Int. J. Pharm.* **224**: 1–18

Hoeller, S., Valenta, C. (2007) Effect of selected fluorinated drugs in a 'ringing' gel on rheological behaviour and skin permeation. *Eur. J. Pharm. Biopharm.* **66**: 120–126

Moser, K., Kriwet, K., Naik, A. Kulia, Y. W., Guy, R. H. (2001) Passive skin permeation enhancement and its quantification in vitro. *Eur. J. Pharm. Biopharm.* **52**: 103–112

Ongpipattanakul, B., Burnette, R. R., Potts, R. O., Francoeur, M. L. (1991) Evidence that oleic acid exists in a separate phase within stratum corneum lipids. *Pharm. Res.* **8**: 350–354

Paolino, D., Ventura, C. A., Nistico, S., Puglisi, G., Fresta, M. (2002) Lecithin microemulsions for the topical administration of ketoprofen: percutaneous adsorption through human skin and in vivo human skin tolerability. *Int. J. Pharm.* **244**: 21–31

Paul, B. K., Moulik, S. P. (2001) Uses and applications of microemulsions. *Curr. Sci.* **80**: 990–1001

Peltola, S., Saarinen-Savolainen, P., Kiesvaara, J., Suhonen, T. M., Urtti, A. (2003) Microemulsions for topical delivery of estradiol. *Int. J. Pharm* **254**: 99–107

Raney Sameersingh, G., Hope, M. J. (2006) The effect of bilayer and hexagonal H (II) phase lipid films on transepidermal water loss. *Exp. Dermatol.* **15**: 493–500

Touitou, E., Godin, B., Karl, Y., Bujanover, S., Becker, Y. (2002) Oleic acid, a skin penetration enhancer, affects Langerhans cells and corneocytes. *J. Control. Release* **80**: 1–7

Wang, H., Zhang, G., Du, Z., Li, Q., Wang, W., Liu, D., Zhang, X. (2006) Effect of temperature on dynamic rheological behaviour of discontinuous cubic liquid crystal. *J. Colloid Interface Sci.* **300**: 348–353