Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 349 (2008) 269–273
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

The advantage of polymer addition to a non-ionic oil in water microemulsion for the dermal delivery of progesterone

Babette Biruss, Claudia Valenta *

Department of Pharmaceutical Technology and Biopharmaceutics, University of Vienna, Althanstrasse 14, 1090 Vienna, Austria

Received 21 May 2007; received in revised form 6 August 2007; accepted 7 August 2007
Available online 11 August 2007

Abstract

The influence of progesterone on the physicochemical behaviour of the o/w microemulsion consisting of the non-ionic surfactant polyoxyethylene-10-dodecyl ether, tributyrin and water was investigated. Thereby no significant influence could be detected in terms of droplet size, zeta potential, conductivity and pH by progesterone. However the chemical stability of progesterone was insufficient during the storage of 6 months. Therefore, two different polymeric agents, named silicon dioxide and polymeric emulsifier, were added to the progesterone containing microemulsions. These polymers increased the chemical stability of progesterone significantly. Moreover the polymeric additives improved the skin permeation 1.24- and 1.63-fold and decreased the skin retention in relation to the pure microemulsion. The polymer-stabilized progesterone microemulsions are interesting vehicles for skin application of progesterone.

Keywords: Progesterone; Polymeric additives; Microemulsion; Stability; Skin permeation

1. Introduction

Microemulsions are thermodynamically stable o/w or w/o emulsions with droplet sizes in the sub-micron range (Lee et al., 2003). In general, they consist of an oil phase, a surfactant, a cosurfactant and an aqueous phase (Rhee et al., 2001). Surfactants are necessary to minimize the hydrophobic interactions between the phases and maintain stable formulations. Surfactants and oil phase as well as the cosurfactant are able to act as penetration enhancers for transdermal drug delivery (Trotta et al., 1990). This mechanism of action is caused by reducing the barrier properties of the skin by disrupting lipid bilayers within the stratum corneum (Gloor et al., 2003; Lee et al., 2003). The use of a non-ionic surfactant such as *n*-alkyl polyoxyethylene ether does not require a cosurfactant. This is a desirable fact because the cosolvents are often not pharmaceutically acceptable and irritative (Lehmann et al., 2001). The chosen oil tributyrin is skin compatible and widely used in cosmetic and food preparations (Hamdam et al., 1996).

Numerous advantages like transparency, ease of preparation, protection of labile drugs, increase of bioavailability, control of drug release and increase of drug solubility can be associated with microemulsions (Lawrence and Malcolmson, 1993). In general microemulsions exhibit low viscosity (Warisnoicharoen et al., 1999; Valenta and Schultz, 2004). Polymers can be added in order to increase viscosity (Peltola et al., 2003; Valenta and Schultz, 2004; Biruss et al., 2006) as well as to stabilize the formulations (Huang et al., 1987), or to improve the solubilization capacity (Sottmann, 2002).

The transdermal delivery of progesterone would provide numerous advantages compared to other routes of administration, especially concerning metabolism, negative systemic side effects, dosage and patient compliance (Biruss et al., 2006; Biruss and Valenta, 2006, 2007). For the present study a cosurfactant-free o/w microemulsion with tributyrin as oily component was selected to incorporate progesterone. In order to gain information about the physicochemical properties following data should be obtained: droplet size, zeta potential, conductivity, pH values and phase inversion temperature. The aim was to improve the chemical stability of progesterone and to increase the skin permeation by addition of silicon dioxide or polymeric emulsifier to the microemulsion.

* Corresponding author. Tel.: +43 1 4277 55 410; fax: +43 1 4277 9554.
E-mail address: claudia.valenta@univie.ac.at (C. Valenta).

0378-5173/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2007.08.003

2. Materials and methods

2.1. Materials

Progesterone, polyoxyethylene-10-dodecyl ether and tributyrin were obtained from Sigma (St. Louis, USA). Silicon dioxide (Aerosil) was purchased from ACM (Au). Polymeric emulsifier (Pemulen TR 1) was donated from Noveon GmbH (Ge).

2.2. Preparation of the microemulsion

Based on preliminary tests such as optimal skin feeling and visual expertise a microemulsion consisting of 2.50 g polyoxyethylene-10-dodecyl ether, 0.34 g tributyrin, 7.20 g distilled water was chosen for investigation. In the progesterone containing microemulsion a final drug concentration of 1% (w/w) was used. For the preparation the drug was mixed with oil and surfactant and heated up to about 60 °C. The resulting mixtures were carefully titrated by hot distilled water and stirred. At higher temperatures (above 70 °C) milky emulsions were observed. At about 45 °C the cloudy emulsions turned into isotropic, completely transparent microemulsions.

2.2.1. Gelified microemulsions

After preliminary viscosity measurements 2% (w/w) of polymeric emulsifier and 6% (w/w) of silicon dioxide were added directly to the microemulsions by gentle stirring in order to receive optimal applicable semisolid formulations. The swelling time was 24 h. All vehicles were transparent with the exception of silicon dioxide containing preparations.

2.3. Physicochemical characterisation of the non-polymeric microemulsions

2.3.1. Optical microscopy

In a first step the microemulsions with and without progesterone were observed by optical microscope in polarized light at room temperature (Caboi et al., 2005). Thereby the isotropic behaviour of the microemulsion should be verified.

2.3.2. Droplet size

The droplet size of the microemulsions with and without progesterone was analysed by laser diffraction with a Zetasizer Nano ZS (Malvern Instruments). Three parallel measurements were at least performed at 25 °C. Subsequently the average size was calculated. The polydispersity index (PDI) describes the homogeneity of the samples. Low values indicate homogenous vesicles, PDI values larger than 0.5 indicate a higher heterogeneity.

2.3.3. Zeta potential

As further characterisation parameter the zeta potential values were analysed by laser Doppler electrophoresis with a Zetasizer Nano ZS (Malvern Instruments) over an observation interval of 4 weeks. The measurements were calculated by Helmholtz–Smoluchowski equation.

2.3.4. Conductivity

In order to verify if the phase behaviour of the microemulsion the conductivity was evaluated by the Zetasizer Nano ZS.

2.3.5. Micro-differential scanning calorimetry (micro-DSC)

In order to gain information about the microstructure of the microemulsion micro-DSC measurements of the non-polymeric microemulsions with and without progesterone were performed (Setaram III, F). Nitrogen was used as purge gas. Approximately 400 mg of sample were weighed precisely into a batch cell. As reference an air filled batch cell was used. After an equilibration time of 20 min a heating procedure from 80 °C to 20 °C was performed and afterwards a cooling procedure at the same temperature range with a scanning rate of 1 K/min was performed.

2.4. Chemical stability

Beside the physicochemical properties and the skin permeation, the chemical stability of the investigated drug in the vehicle plays a major role. Therefore the drug content was analysed at defined time intervals for an observation period of 6 months. During the observation period the formulations were stored in tubes under room temperature to stimulate patient usage conditions (Biruss et al., 2006).

For the experiment a defined amount of each formulation containing progesterone was dissolved in 1 ml methanol and centrifuged for 6 min. Twenty microlitres were analysed by HPLC. The comparison of the resulting chromatograms demonstrated that the main peak decreased and degradation products occurred within the observation period as additional peaks. The progesterone content was analysed on the day of preparation and quoted as 100%.

2.5. Rheological experiments

For characterisation of the drug-free and the progesterone containing microemulsions without polymers viscosity measurements were performed on a Haake MARS rheometer. As tool a thermostatically controlled plate/plate with 60 mm in diameter (PP60/Ti) was used. For characterisation of the drug-free and the progesterone containing microemulsions with polymers viscosity measurements were performed on a Haake rheometer Rotovisco RT 20 (Haake, Karlsruhe, Germany, thermo controller Haake F6/8). As tool a thermostatically controlled cone/plate with 35 mm in diameter and 2° angle (C35/2Ti) was used.

The plate temperature was 20 ± 1.5 °C and the applied sample amount was about 1 g.

The following parameters were used: controlled rate (CR) modus, $\gamma = 1$–$100\,s^{-1}$; 100–$1\,s^{-1}$; 20 ± 1.5 °C. In brief, CR modus means that a controlled shear rate is applied. In order to receive a better comparison between the results of all formulations the viscosity values at a G_p of $4.9\,s^{-1}$ were compared. All rheological experiments were performed in triplicate.

2.6. Skin permeation

The diffusion of progesterone was investigated using Franz-type permeation cells thermostated at 32 °C (Permegear, US). The excised porcine skin prepared by a dermatome (GB 228R, Aesculap, Ge) set at 1.2 mm was mounted in the diffusion cell with the stratum corneum uppermost. An amount of 0.6 g of each formulation was applied. The receptor compartment consisted of 2 ml propylene glycol/water (40 + 60 w/w) and was constantly stirred by a magnetic bar. At defined time intervals 200 μl of receptor media were analysed and replaced by new medium. For all formulations minimum three parallel experiments were performed. The progesterone content was analysed by HPLC as previously reported (Biruss and Valenta, 2006).

2.7. Skin retention

Skin retention experiments were performed in order to analyse the content of progesterone stored in skin after 48 h of diffusion. At the end of the experiment the skin samples were carefully washed with distilled water and methanol on both sides and carefully dried. Afterwards a defined amount of methanol was added to each piece of skin. The samples were vortexed for 10 min and stirred overnight.

2.8. HPLC analysis

The progesterone content of skin diffusion, skin retention and chemical stability experiments was analysed by HPLC (Perkin-Elmer, US) according to an established method (Biruss and Valenta, 2006) using a Nucleosil 100 5 C-18 column (240 mm × 4.6 mm) as stationary phase and methanol/water (90 + 10 w/w) as mobile phase. The detection wavelength was 240 nm. The retention time was about 4.2 min. The flow rate was 1 ml/min. Twenty microlitres of each sample were injected. Calibration curves were calculated on the basis of peak area measurements. They were generated with a correlation coefficient of 1.0. The concentration range for progesterone was between 7.73 μg/ml and 123.7 μg/ml.

2.9. Statistical data analysis

Results are expressed as the means of at least three experiments ± S.D. Statistical data analysis was performed using the Student's *t*-test with $p < 0.05$ as a minimal level of significance. XLSTAT version 5.2. software was used for statistical analyses.

3. Results and discussion

3.1. Formulations

For the present investigations one o/w microemulsion consisting of a non-ionic surfactant, distilled water and tributyrin was selected to incorporate progesterone. Volatile, transparent preparations were obtained. Two additives, silicon dioxide and polymeric emulsifier were chosen, on one hand to improve adhesion and applicability on skin and on the other hand to increase the viscosity and the chemical stability of progesterone. After direct addition of these additives semisolid formulations were received.

Table 1
Physicochemical characterisation of the non-polymeric microemulsions with and without progesterone measured at the day of preparation; $n = 3$

Parameter	Microemulsion without progesterone	Microemulsion with progesterone
Average droplet size (nm)	5.41 ± 0.02[a]	5.50 ± 0.14[b]
Zeta potential (mV)	−9.34 ± 0.19	−13.24 ± 5.97
Conductivity (mS/cm)	0.33 ± 0.002	0.32 ± 0.003
pH value	5.54	5.72
Phase inversion temperature (°C)	45.5 ± 0.4*	44.5 ± 0.4*

[a] PDI (polydispersity index) = 0.27–0.29.
[b] PDI = 0.25–0.27.
* Means a significant difference.

3.2. Physicochemical characterisation of the non-polymeric microemulsions

The influence of progesterone on different physicochemical properties was analysed. The isotropic behaviour of the microemulsion could be proven by polarisation microscopy, where no changes were seen by the incorporation of progesterone.

For all these investigations it was looked whether progesterone had an influence. In Table 1 it can be seen that this is not the case. The droplet sizes are in the same range. This might indicate that the drug is well solubilised and incorporated in the vehicle. The droplet size of microemulsions usually ranges from 10 nm to 100 nm. In comparison the investigated microemulsion is very small. The formation of microemulsions is a spontaneous process. The size of the droplets strongly depends on the composition of the microemulsion. This fact makes the importance of exact investigation of the microemulsion's droplet size more obvious.

It can be seen that progesterone did not influence any of the physicochemical values with exception of the phase inversion temperature.

As further parameter the zeta potential was evaluated. For microemulsions with non-ionic surfactants the zeta potential (Table 1) can be used to analyse the charge of the system. It can be seen that the microemulsional system is negatively charged which might be caused by tributyrin. In order to achieve physical stability information of the microemulsion, the zeta potential was measured over an observation period of 4 weeks. The weekly measured values are summarized in Fig. 1. As seen the zeta potential values in the microemulsion did not change significantly during the observation period of 4 weeks independent of drug content. Thereby it is confirmed that the physical stability of the microemulsion will be high.

The conductivity measurements confirm an oil in water phase behaviour.

Another interesting point is the phase inversion temperature, where the cloudy dispersions turn into completely transparent,

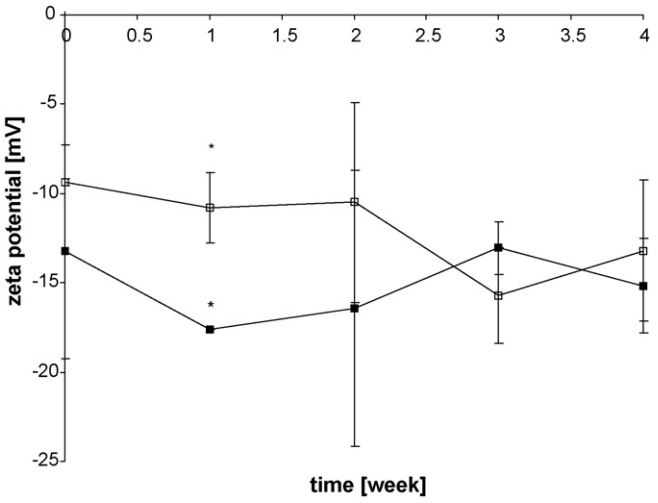

Fig. 1. Values of the zeta potential in the pure microemulsions with and without progesterone after an observation period of 4 weeks in mV; $n = 3$ (□ microemulsion without progesterone; ■ microemulsion with progesterone).

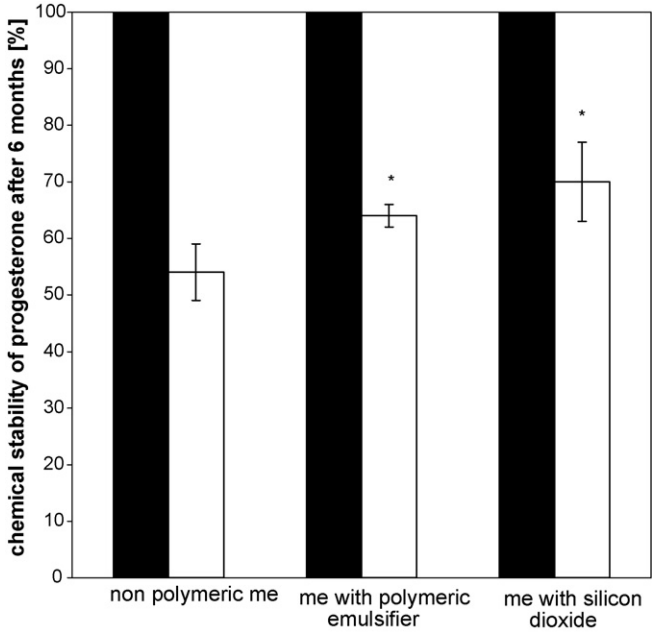

Fig. 2. The chemical stability of progesterone in microemulsions with and without polymeric additives after a storage duration of 6 months (□) in % (w/w); $n = 3$. The amount of progesterone at the day of preparation is quoted as 100% (■).

isotropic, volatile microemulsions. The temperature represented by an exothermic peak (curves not shown) can be seen in Table 1. Progesterone decreased this phase inversion temperature for 1 °C significantly. This value is highly reproducible on the used very sensitive microcalorimeter. Although this is a rather small difference it could be an indication for an interaction between progesterone and the microemulsional structure.

3.3. Chemical stability

The chemical stability of progesterone in the microemulsion was insufficient. As seen (Fig. 2) 46% of progesterone degraded in the pure microemulsion after 6 months. Whereas the addition of polymeric emulsifier increased the stability of progesterone up to 64% and the addition of silicon dioxide increased it to 70% (Fig. 2). The improved stability obtained by the polymeric additives might be caused by an interaction between the microemulsion droplets and the polymers (Huang et al., 1987). With regard to the chemical structure of silicon dioxide it is likely that strong hydrogen bonding between the silanol and siloxan groups of the polymer and the microemulsion leads to a higher chemical stability compared to polymeric emulsifier.

3.4. Rheological investigations

The results of the viscosity measurements are listed in Table 2. The viscosity data of the pure microemulsion is about 0.45 Pa s, independent of whether progesterone is incorporated or not. By addition of polymeric emulsifier or silicon dioxide the viscosity is increased about 50- and 20-fold, respectively compared to the polymer-free microemulsion. By the addition of progesterone the viscosity is further increased in both systems. A reason might be a difference in solubilisation capacity for progesterone induced by the polymers.

3.5. Skin diffusion

The results after 48 h of skin diffusion are summarized in Fig. 3. As indicated the polymers were able to increase progesterone skin diffusion. By addition of silicon dioxide the skin permeation was 1.24-fold and by addition of polymeric emulsifier the skin diffusion was 1.63-fold enhanced after 48 h of diffusion.

The used polymeric emulsifier is a copolymer of acrylic acid modified by long chain (C10–C30) alkyl acrylates cross-linked with allylpentaerythritol. With regard to the chemical structure a reason for the better skin diffusion of polymeric emulsifier compared to silicon dioxide can be seen in its lipophilic structure.

A general reason for the improved skin diffusion by polymeric emulsifier and silicon dioxide might be a possible increase of progesterone solubility by the polymers (Biruss and Valenta, 2006). Moreover the skin adhesion is improved by the polymers.

Table 2
Comparison of the viscosity of all formulations at a G_p of 4.9 s^{-1} measured at 20 ± 1.5 °C; $n = 3$

	Pure microemulsion ± S.D.	Polymeric emulsifier ± S.D.	Silicon dioxide ± S.D.
η (Pa s) (progesterone)	0.45 ± 0.09	29.00 ± 0.14*	18.31 ± 2.84*
η (Pa s) (placebo)	0.43 ± 0.07	21.90 ± 6.00*	8.50 ± 4.76*

* Means a significant difference.

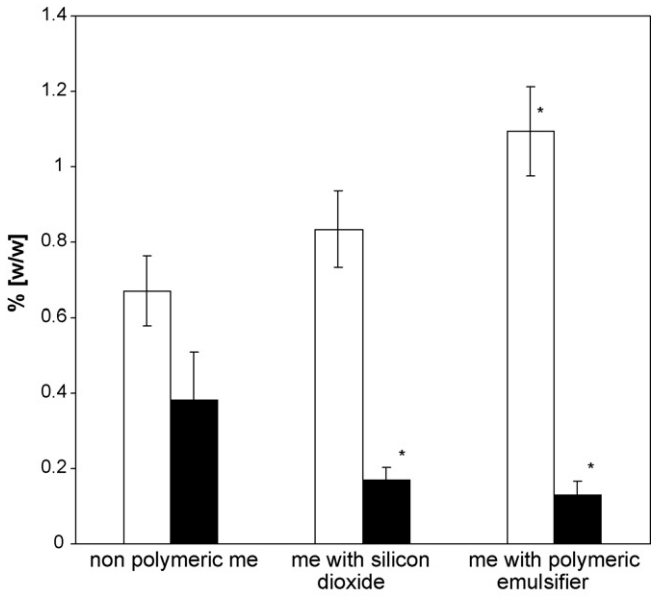

Fig. 3. Permeated amount and skin retention of progesterone containing microemulsions with and without polymers in % (w/w) after 48 h of diffusion; $n = 3$ (□ permeated amount; ■ skin retention).

In contrast the more progesterone permeates the less progesterone is retained in skin (Fig. 3).

4. Conclusion

It can be concluded that the addition of silicon dioxide and polymeric emulsifier to an o/w microemulsion increased the chemical stability of progesterone and increased the skin permeation. Therefore these systems are interesting multifunctional vehicles for the dermal application of progesterone. Indeed to dispose a general rule for other different polymers would be too venturous because the skin permeation and chemical stability behaviour of a dermal formulation depend on numerous parameters such as type of polymer, composition of microemulsion and drug. Therefore different formulations with different polymers should be individually investigated in further studies.

References

Biruss, B., Valenta, C., 2006. Skin permeation of different steroid hormones from polymeric coated liposomal formulations. Eur. J. Pharm. Biopharm. 62, 210–219.

Biruss, B., Valenta, C., 2007. Comparative characterisation of the physicochemical behaviour and skin permeation of extruded DPPC liposomes modified by selected additives. J. Pharm. Sci. 96, 2171–2176.

Biruss, B., Kählig, H.P., Valenta, C., 2006. Evaluation of an eucalyptus oil containing topical drug delivery system for selected steroid hormones. Int. J. Pharm..

Caboi, F., Lazzari, P., Pani, L., Monduzzi, M., 2005. Effect of 1-butanol on the microstructure of lecithin/water/tripalmitin system. Chem. Phys. Lipids 135, 147–156.

Gloor, M., Hauth, A., Gehring, W., 2003. O/W emulsions compromise the stratum corneum barrier and improve drug penetration. Pharmazie 58, 709–715.

Hamdam, S., Faujan, B.H.A., Laili, C.R., Ahmad, W.B.W., 1996. Water/food flavour microemulsion systems. J. Agric. Food Chem. 44, 962–963.

Huang, J.S., Fetters, L.J., Dozier, W.D., Sung, J., 1987. Stabilization of microemulsions by polymers. Polym. Mater. Sci. Eng. 57, 965–969.

Lawrence, M.J., Malcolmson, C., 1993. A comparison of the incorporation of model steroids into non-ionic micellar and microemulsion systems. J. Pharm. Pharmacol. 45, 141–143.

Lee, P.J., Langer, R., Shastri, V.P., 2003. Novel microemulsion enhancer formulation for simultaneous transdermal delivery of hydrophilic and hydrophobic drugs. Pharm. Res. 20, 264–269.

Lehmann, L., Keipert, S., Gloor, M., 2001. Effects of microemulsions on the stratum corneum and hydrocortisone penetration. Eur. J. Pharm. Biopharm. 52, 129–136.

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.

Rhee, J.S., Choi, J.G., Park, E.S., Chi, S.C., 2001. Transdermal delivery of ketoprofen using microemulsions. Int. J. Pharm. 228, 161–170.

Sottmann, T., 2002. Solubilization efficiency boosting by amphiphilic block co-polymers in microemulsions. Curr. Opin. Colloid Interface Sci. 7, 57–65.

Trotta, M., Gasco, M.R., Pattarino, F., 1990. Diffusion of steroid hormones from oil-in-water microemulsions: influence of the cosurfactant. Acta Pharm. Technol. 36, 226–231.

Valenta, C., Schultz, K., 2004. Influence of carrageenan on the rheology and skin permeation of microemulsion formulations. J. Control. Release 95, 257–265.

Warisnoicharoen, W., Lansley, A.B., Lawrence, M.J., 1999. Nonionic oil-in-water microemulsions: the effect of oil type on phase behaviour. Int. J. Pharm. 198, 7–27.