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Evaluation of an eucalyptus oil containing topical drug delivery system for selected steroid hormones

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Abstract

In the present study the permeation and the chemical stability of 17-β-estradiol, progesterone, cyproterone acetate and finasteride incorporated in an eucalyptus oil containing microemulsion system have been investigated. The formulations contained 1% (w/w) of the steroid hormones. Self diffusion coefficients determined by pulsed-field-gradient spin echo NMR spectroscopy were used to characterise the microemulsion. From these results a bicontinous structure is proposed for the multicomponent system. However a correlation between the self diffusion of the hormones in the vehicle and the transdermal flux was not indicated. Explanations for this were self assembling, formation of aggregates between the components of the microemulsion and drugs and different effects because of different solubility of the drugs. By addition of certain polymers the skin permeation rates could be improved with exception of cyproterone acetate. Beside standard diffusion experiments, the residual drug content in the skin was investigated. Drug stability was monitored by analysing the steroid hormone content in the different formulations over an observation period of 6 weeks and could be improved by polymers. In addition, viscosity measurements were performed. They indicated an influence of the polymers and drugs on the viscosity in all formulations.

Keywords: Steroid hormones; Microemulsion; Skin permeation; NMR; Chemical stability; Viscosity

1. Introduction

The dermal and transdermal route offer numerous advantages compared to other routes of administration (Burgess et al., 2005). Especially the topical administration of steroid hormones like 17-β-estradiol, progesterone, cyproterone acetate and finasteride would mean a great benefit concerning metabolism, negative systemic side effects and dosage (Biruss and Valenta, 2006). The mechanism of action involves the binding to specific intracellular receptors in certain areas of the skin (Whiting et al., 2000).

Microemulsions are of pharmaceutical interest to act as transdermal, oral, parenteral, pulmonary and ocular drug delivery systems by interacting with several membranes (Lawrence and Rees, 2000). They are thermodynamically stable, isotropic dispersions of oil, water and surface active agents (Lapasin et al., 2001). Often co-surfactants are present. Further reported advantages associated with microemulsions include their ease of preparation, protection of labile drugs, increase of bioavailability, control of drug release and increase of drug solubility (Lawrence and Rees, 2000). Microemulsions are completely transparent, have a droplet size <0.15 μm and are defined as o/w and w/o. Beside a bicontinuous type of microemulsions exists (Rhee et al., 2001). The formation of the type of microemulsion depends on the used surfactant and oil (Kreilgaard, 2002; Yaghmur et al., 2002). Both as well as the cosurfactant like ethanol can be able to act as penetration enhancers (Trotta et al., 1990; Lawrence and Rees, 2000). This mechanism is reported to be 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).

In the present study a microemulsion system containing the non-ionic surfactant Brij-30, ethanol as cosurfactant and eucalyptus oil was used as basic vehicle (Acharya et al., 2001; Mitra and Paul, 2005). The major component of eucalyptus oil is eucalyptol, a 1,3,3-trimethyl-2-oxabicyclo[2,2,2]-octane.

Abbreviations: Brij-30, polyoxyethylene(4) ether; DLS, dynamic light scattering; *J*, fluxes; K_p, permeation coefficients; PEO, poly-ethyleneoxide; PFG, pulsed field gradient

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Eucalyptus oil is volatile, colourless and frequently used as flavouring agent or expectorant. Furthermore, it is a multifunctional component that possesses bactericidal, antifungal (Shahi et al., 2000) as well as permeation enhancing properties. In the present study 17-β-estradiol, progesterone, cyproterone acetate and finasteride were incorporated into an eucalyptus oil containing microemulsion. The resulting volatile transparent formulations should be characterised by generating self diffusion coefficients of the individual components of the vehicle as well as of the hormones by NMR. In a next step the formulations should be investigated in terms of drug release, drug retention, chemical stability of the incorporated hormones and viscosity. Moreover it was the question, whether polymers could be able to improve physicochemical behaviours as has been demonstrated for polymer coated phospholipid liposomes (Biruss and Valenta, 2006).

2. Materials and methods

2.1. Materials

17-β-Estradiol, progesterone, polyoxyethylene(4) lauryl ether (Brij-30) were purchased from Sigma (St. Louis, USA). Cyproterone acetate was a generous gift from Intendis (Au). Finasteride was purchased from Kemprotec (UK). Eucalyptus oil and silicon dioxide (Aerosil) were obtained from ACM (Au). Polycarbophil (Noveon AA1) and polymeric emulsifier (Pemulen TR 1) were donated from Noveon GmbH (Ge).

All other chemicals were of analytical reagent grade and used without any further purification.

2.2. Formulations

2.2.1. Microemulsion

The modified microemulsion was based on a previously described formulation (Acharya et al., 2001) and consisted of 22% (w/w) polyoxyethylene(4) lauryl ether (Brij-30), 22% (w/w) ethanol, 45% (w/w) eucalyptus oil and 10% (w/w) distilled water. For all hormones a final concentration of 1% (w/w) was established, the hormones were: 17-β-estradiol, progesterone, cyproterone acetate and finasteride. Firstly, for each formulation the hormones were completely dissolved in ethanol. Afterwards Brij-30 and eucalyptus oil were added by constant stirring. Finally, the solutions were carefully titrated by distilled water and stirred until clear formulations resulted.

2.2.2. Microemulsions with polymers

Defined amounts of microemulsions containing the different drugs were gelified by polycarbophil and polymeric emulsifier in a final concentration of 2% (w/w) and silicon dioxide in a final concentration of 6% (w/w). The excipients were added directly to the microemulsions by gentle stirring. After a swelling time of about 24 h the resulting transparent semisolid preparations were stored in suitable vessels.

Eucalyptus oil and ethanol might cause inflammatory reactions, toxicity and should not be used for children. In further investigations in vitro toxicity studies should be performed.

2.3. NMR diffusion experiments

Self diffusion measurements were performed on a Bruker Avance DRX 600 NMR spectrometer (Bruker BioSpin GmbH Rheinstetten, Germany) operating at a frequency of 600.13 MHz for ^{1}H by using a 5 mm triple inverse probe (TBI) with triple axis gradient coils, and an Acustar II gradient amplifier (3×10 A, maximum amplitude 50 Gcm^{-1} for x or y, 65 Gcm^{-1} for z). For spectrometer stability reasons the 10% (w/w) distilled water in the microemulsion formulation was replaced by D_2O. The diffusion coefficients at a temperature of 300 K were derived from the signal attenuation in a series of 16 pulsed field gradient (PFG) stimulated spin echo spectra with increasing gradient amplitudes g (from 3 to 95% of maximum amplitude) by constant diffusion delay Δ (e.g. 100 ms for fast decreasing or 300 ms for slow decreasing signals) as follows (Wu et al., 1995):

$$I = I_0 \exp[-D\gamma^2 g^2 \delta^2 (\Delta - \delta/3 - \tau/2)]$$

where I is the observed signal intensity, I_0 the unattenuated signal intensity, D the diffusion coefficient, γ the gyromagnetic ratio of the observed nucleus ^{1}H, δ the length of the gradient (1 ms), and τ is the time between bipolar gradients. To minimize thermal convection artefacts, only transverse PFGs were used. All the processing and analysis was done within the Bruker software Topspin, version 1.3 (Bruker BioSpin GmbH Rheinstetten) by using the T1/T2 package for calculating the self diffusion coefficients. All experiments were repeated at least 5 times, the accuracy was within ±4%.

2.4. Skin preparation

Porcine abdominal skin which has a similar lipid composition to human skin (Bartek et al., 1972) was shaved. Then the subcutaneous fat layer was carefully removed. The final preparation was carried out with a dermatome (GB 228R, Aesculap, Ge) set at 1 mm. The porcine skin was stored in a freezer at −20 °C and thawed 2 h before use.

2.5. Diffusion cell preparation

The permeation of all hormones was investigated by the use of Franz-type diffusion cells (Permegear, US). For this purpose 0.6 g of each formulation was applied on a permeation area with a surface of about 1.13 cm^2. The receptor compartment was filled with 2 ml propylene glycol/ water (40 + 60, w/w) thermostated at 32 °C and stirred by a magnetic bar. The excised skin was mounted in the Franz-type diffusion cell, stratum corneum uppermost, with the dermal side close to the receptor compartment. Samples of 200 μl were removed at defined time intervals for analysis and replaced by fresh receptor medium for 48 h. Three parallel experiments were at least performed for each formulation.

Additionally the fluxes J [μg cm^{-2} h^{-1}] and the permeation coefficients K_p [cm h^{-1}] of all formulations were calculated by the use of the concentration of the drug in the vehicle C_V

[$\mu g\,cm^{-3}$] and compared.

$$J = K_p \times C_V$$

2.6. Skin retention

Furthermore experiments were performed in order to analyse the content of steroid hormones in skin after 48 h of diffusion. At the end of the experiment the skin samples were washed up with water and methanol on both sides and carefully dried. After this procedure a defined amount of methanol was added to each piece of skin. The samples were vortexed for 10 min and stirred overnight. After centrifugation the samples were analysed by HPLC.

2.7. Chemical stability

In order to characterize the dependence of the chemical stability of the drugs on the different used vehicles stability studies were performed. All formulations were stored in tubes under room temperature for 6 weeks. The drug content of a certain amount of each formulation was analysed at the day of preparation (starting point). This value was quoted as 100%. Afterwards samples were taken weekly.

Therefore a defined amount of formulation was dissolved in 1 ml methanol and centrifuged for 6 min. Twenty microliters were analysed by HPLC.

2.8. HPLC analysis

All samples were analysed for their drug content by HPLC (Perkin-Elmer, US) consisting of an automatic autosampler ISS-200 (Perkin-Elmer) at a flow rate of 1 ml/min of mobile phase, peak detection by UV (Perkin-Elmer, LC 235 diode array) and a pump (Perkin-Elmer, series 200 LC pump). All stationary phases were provided from ARC-Seibersdorf GmbH (Au).

The analytic procedures for all used hormones were recently reported (Biruss and Valenta, 2006).

2.9. Rheological experiments

The rheological properties of the drug containing microemulsions with the different polymers have been examined and compared. All oscillatory experiments were performed at 20 °C on a Haake rheometer Rotovisco RT 20 (Haake, Karlsruhe, Germany, thermo controller Haake F6/8) using a cone and plate C 35°/2. About 1 g sample was applied. By this modulus the induced response (strain) is measured when a sinusoidal stress is applied to the sample. After the identification of the linear viscoelastic region, samples were investigated over a frequency of 0.1–10 Hz (ν). The obtained parameters are the elastic modulus G', the viscous modulus G'' and the dynamic viscosity η' which are calculated by the following formula.

$$G' = G\,\cos(\delta)$$
$$G'' = G\,\sin(\delta)$$
$$\eta' = G''/\omega$$

ω is the angular velocity of oscillatory stress which is related to the oscillatory frequency by the relationship $\omega = 2\pi\nu$. The related phase angle is expressed as δ.

2.10. 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.

3. Results

3.1. Formulations

Microemulsions consisting of ethanol, Brij-30, eucalyptus oil and demineralised water were prepared with incorporated 17-β-estradiol, progesterone, cyproterone acetate and finasteride at a final concentration of 1% (w/w). In all cases clear optical transparent and volatile microemulsions resulted with odour of eucalyptus oil and semisolid formulations emerged by adding polycarbophil, polymeric emulsifier and silicon dioxide, respectively. All resulting products were optical transparent too.

3.2. Self diffusion coefficients in the pure microemulsion

Only the free and drug loaded pure microemulsions without polymers were investigated by diffusion NMR measurements. The ^{1}H NMR spectra of these formulations are well resolved and one or more characteristic signals could be easily assigned to the individual components in order to extract their diffusion coefficients from the pulsed field gradient (PFG) stimulated spin echo experiments. For ethanol the quartet signal of the CH_2 group at 3.765 ppm or the triplet signal of the CH_3 group at 1.33 ppm, for the oil component the signals of the methyl groups from eucalyptol at 1.37 or 1.17 ppm, and for the surfactant the ethylene signals between 3.87 and 3.57 ppm or the CH_3 group at 1.06 ppm were used. The analysis of the water signal at 4.6 ppm requires special considerations, as both the alcohol and the surfactant contain an exchangeable proton. But calculating the diffusion coefficient for water D_W by taking into account all this individual exchange contributions to the experimentally observed value (Nilsson and Lindman, 1983) results due to the high mole fraction of D_2O only in a very small increase in D_W of about 1%.

In Table 1 the diffusion coefficients obtained by PFG stimulated spin echo experiments are summarized for all components within the microemulsion as well as for the neat liquids. First of all the measured D values for the main components are within the experimental error independent from the dissolved very low concentrated steroids. The characterisation of the microstructure of the formulation can be performed based on the relative self diffusion coefficients D_{rel} of the water and oil components which is given by the fraction of D/D_0, where D_0 denotes the self diffusion coefficient of the pure solvent and D the corresponding value in the microemulsion. As the derived values (Table 1) with D_{rel} for water = 0.21 and D_{rel} for oil = 0.32 are in the same

Table 1
Self diffusion coefficients of the microemulsion components (D in the pure microemulsions, D_0 of the neat liquids, $D_{rel} = D/D_0$)

	D [m^2 s^{-1}]	D_0 [m^2 s^{-1}]	D_{rel}
Water	3.9×10^{-10}	1.9×10^{-9}	0.21
Eucalyptus oil	2.9×10^{-10}	9.1×10^{-10}	0.32
Ethanol	4.1×10^{-10}	1.1×10^{-9}	0.37
Polyoxyethylene(4) lauryl ether (free) (micellized)	2.2×10^{-10}	9.2×10^{-11}	
	8.3×10^{-11}	9.6×10^{-12}	

order of magnitude, a bicontinuous structure with both solvents forming domains that extend over macroscopic dimensions can be suggested (Lindman et al., 1999).

In addition the polar domain is also defined by the co-surfactant ethanol, which exhibits a normalised diffusion coefficient in the same range ($D_{rel} = 0.37$). The diffusion of Brij-30 shows a biexponential behaviour (Table 1) which was already observed for other poly-ethyleneoxide (PEO) containing polymer surfactants (Kählig et al., 2005; Momot et al., 2003). The ratio of the fast free PEO block compared to the slow micellar bound PEO block is about 1–1.4. The numerical values of these two diffusion coefficients (Table 1) are significantly higher compared to that of the neat liquid, which can be attributed to the good solubility of Brij-30 in the low viscous co-surfactant ethanol.

The NMR diffusion analysis of the steroid hormones is hampered by the very low amount of only 1% (w/w) in the formulations. Nevertheless, undisturbed resonance signals could be found mainly in the chemical shift region of the olefinic protons or in case of 17-β-estradiol in the chemical shift region of the aromatic protons, respectively (Fig. 1).

The diffusion coefficients of the investigated steroids in the microemulsion calculated from the signal decay of the PFG spin echo experiments (Fig. 2) are given in Table 2. The derived values are different for the individual hormones. In order to classify their observed diffusion behaviour in the microemulsion environment, reference diffusion measurements were performed with solutions of the steroids in deuterated methanol (concentration 5 mM). As expected, the diffusion values in methanol D_m are significantly increased about 5–8 times, as the viscosity of the solution is much lower. Besides the viscosity the self diffusion should correlate with the molecular size giving the lowest value for the bigger structures. But comparing the experimental diffusion coefficients of the steroids with their molecular volume (Table 2) 17-β-estradiol does not fit into the row of the three others. By having the smallest molecular volume its D_m value is close to the largest structure in the present investigation, namely finasteride.

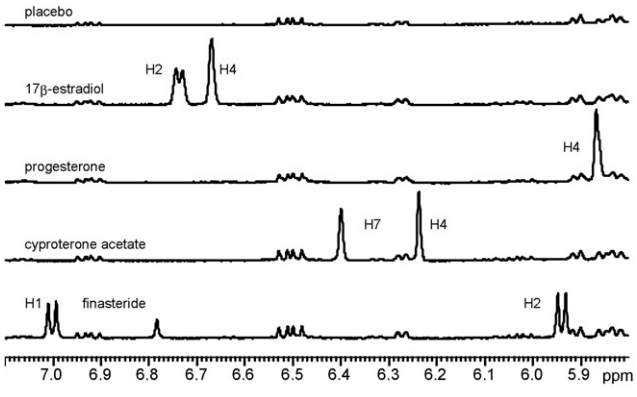

Fig. 1. Expansions of ^{1}H NMR spectra from the free (top trace) and drug loaded pure microemulsiones. The assigned signals of the steroids were used for the analysis of the self diffusion experiments.

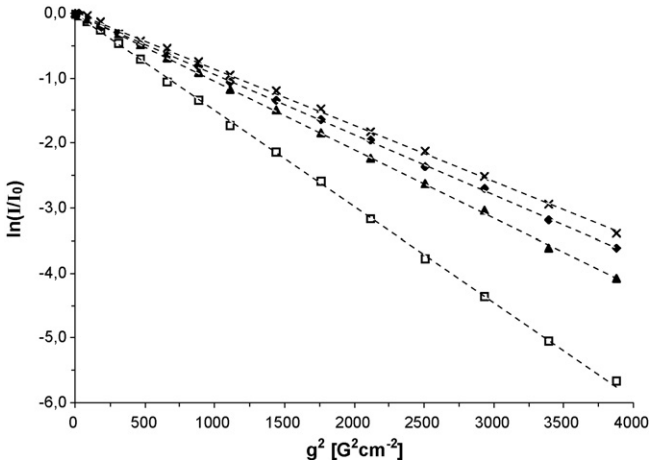

Fig. 2. Pulsed field gradient (PFG) spin echo decay of 17-β-estradiol (♦), progesterone (■), cyproterone acetate (▲) and finasteride (×) in the pure microemulsion (I echo amplitude in the presence, I_0 in the absence of the gradient pulse, g strength of the gradient pulse).

Recently this diffusion anomaly was related to the forming of cluster structures by intermolecular hydrogen bonding between molecules containing polar groups (Shikii et al., 2004, 2005). In these studies the steroids with a hydroxyl or carboxyl group were shown to exhibit a much slower diffusion in methanol as could be expected based solely on their molecular volume. The same effect seems to be present in our study for 17-β-estradiol, the only steroid with a hydroxyl group, although we used different experimental conditions, namely two times lower concentration and higher temperature. Moreover, we can assign the observed diffusion behaviour in methanol directly to the microemulsion formulations, by finding the same sequence of the D values for the steroids though on a 5–8 times lower diffusion scale (Table 2, Fig. 2). This indicates, that the steroid hormones are dissolved in the hydrophilic phase formed by the co-surfactant ethanol and water. Essentially, the PFG derived self diffusion coefficients of the steroids in the microemulsion seem to correlate with their molecular volume, taking into account the afore men-

Table 2
Self diffusion coefficients and the molar volume of the steroids (D in the pure microemulsions, D_m in deuterated methanol, $D_{rel} = D/D_m$, M_V molar volume)

	D [m^2 s^{-1}]	D_m [m^2 s^{-1}]	D_{rel}	M_V [cm^3]
17-β-Estradiol	1.1×10^{-10}	8.6×10^{-10}	0.13	232.6
Progesterone	1.9×10^{-10}	1.0×10^{-9}	0.19	288.9
Cyproterone acetate	1.3×10^{-10}	8.8×10^{-10}	0.15	327.0
Finasteride	9.5×10^{-11}	7.4×10^{-10}	0.13	349.5

tioned aggregation phenomenon of 17-β-estradiol. In addition normalized diffusion coefficients D_{rel} can be calculated from the ratio of D in the formulation divided by D_m in pure methanol, which serves in this case as reference system (Table 2). The lowest values were found for 17-β-estradiol and for finasteride, although probably resulting from different effects. Besides the self assembling, the low diffusion coefficient can be attributed to an additional interaction of the polar steroid 17-β-estradiol with the polar PEO part of the surfactant. In the case of finasteride, this significant reduction in the self diffusion coefficient can be interpreted in terms of an increased solubility in the lipophilic phase of the vehicle compared to the other steroids.

3.3. Skin permeation

3.3.1. Pure microemulsion

A comparison of the diffusion of 17-β-estradiol, progesterone, cyproterone acetate and finasteride (Fig. 3, Table 3) in the pure microemulsion showed that finasteride achieved the highest significantly cumulative permeation rates in μg/cm^2 after 48 h of diffusion. The amount was about 2-fold higher compared to the other tested hormones. However, the diffusion rates of 17-β-estradiol, progesterone and cyproterone acetate were not significantly different but in the same range. The rank order of the cumulative amounts permeated after 48 h of diffusion was finasteride > progesterone > 17-β-estradiol > cyproterone acetate.

3.3.1.1. Correlation between transdermal permeation rate and self diffusion coefficients. All hormones were completely dissolved in the vehicle and the derived self diffusion coefficients by NMR are different for the individual steroids. A correlation of transdermal flux with these self diffusion values could not be established easily. Only in the case of finasteride the reduced normalized diffusion coefficient D_{rel}, due to an increased interaction with the apolar phase, would support the observed highest permeation rate through porcine skin.

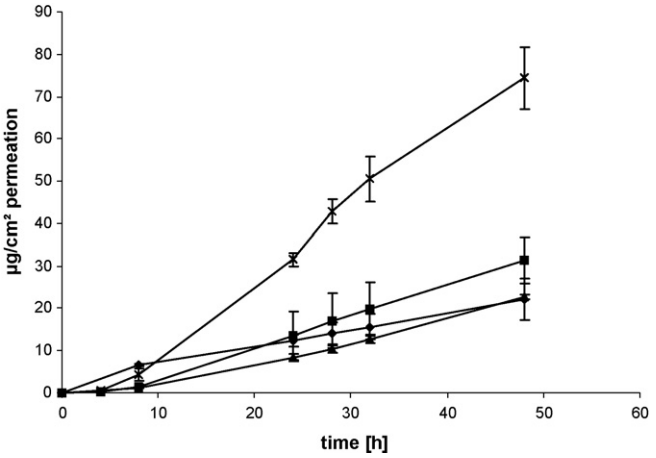

Fig. 3. Release profile of 17-β-estradiol, progesterone, cyproterone acetate and finasteride in microemulsions through porcine skin in μg/cm^2. 17-β-Estradiol (♦), progesterone (■), cyproterone acetate (▲) and finasteride (×). Indicated values are means (±S.D.) of three experiments.

3.3.2. Microemulsions with polymeric emulsifier

Polymeric emulsifier is a more lipophilic matrix than polycarbophil. The results so far (Fig. 4) clearly correspond with the released cumulative hormone amounts after 48 h. This polymer obtained the best permeation results for 17-β-estradiol.

3.3.3. Microemulsions with polycarbophil

Formulations with polycarbophil showed similar results as from the pure microemulsions (Fig. 5) but higher perme-

Table 3
Comparison of the fluxes (J), permeation coefficients (K_p), permeated amounts after 48 h of diffusion and residual drug content in the skin for all formulations; $n = 3$

	Pure microemulsions	Microemulsions with silicon dioxide	Microemulsions with polymeric emulsifier	Microemulsions with polycarbophil
17-β-Estradiol				
J [μg cm^{-2} h^{-1}]	0.4384	0.5397	0.6729	0.6173
K_p [cm h^{-1}]	0.0073	0.0090	0.0112	0.0103
Permeated amount at 48 h [μg/cm^2]	22.06 ± 4.95	26.79 ± 4.65	32.58 ± 1.97	31.25 ± 7.08
Residual drug in skin [%]	0.28 ± 0.08	0.18 ± 0.05	0.26 ± 0.02	0.22 ± 0.06
Progesterone				
J [μg cm^{-2} h^{-1}]	0.6838	0.5064	0.7800	0.8632
K_p [cm h^{-1}]	0.0114	0.0084	0.0130	0.0144
Permeated amount at 48 h [μg/cm^2]	31.26 ± 5.45	23.72 ± 7.86	36.00 ± 2.40	45.63 ± 5.18
Residual drug in skin [%]	0.29 ± 0.01	0.17 ± 0.03	0.33 ± 0.08	0.40 ± 0.04
Cyproterone acetate				
J [μg cm^{-2} h^{-1}]	0.4748	0.2415	0.4826	0.1730
K_p [cm h^{-1}]	0.0079	0.0040	0.0080	0.0029
Permeated amount at 48 h [μg/cm^2]	22.73 ± 0.78	11.24 ± 2.13	22.30 ± 0.78	8.05 ± 0.12
Residual drug in skin [%]	0.28 ± 0.05	0.13 ± 0.01	0.17 ± 0.02	0.28 ± 0.1
Finasteride				
J [μg cm^{-2} h^{-1}]	1.6497	1.8600	2.2053	3.2612
K_p [cm h^{-1}]	0.0275	0.0310	0.0368	0.0544
Permeated amount at 48 h [μg/cm^2]	74.42 ± 7.31	93.15 ± 11.91	101.31 ± 1.15	158.03 ± 9.95
Residual drug in skin [%]	0.53 ± 0.01	0.29 ± 0.06	0.54 ± 0.17	0.54 ± 0.05

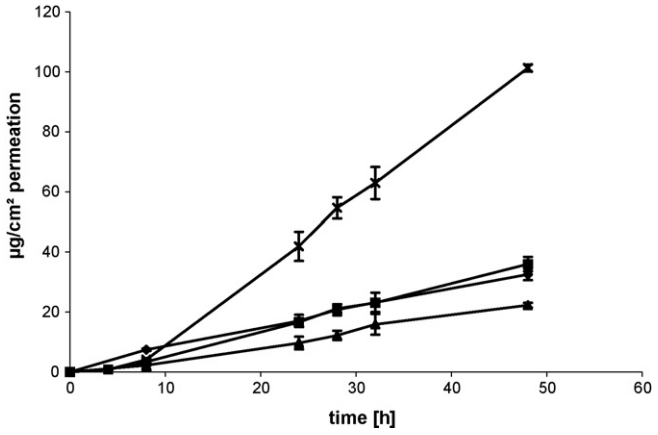

Fig. 4. Release profile of 17-β-estradiol, progesterone, cyproterone acetate and finasteride in microemulsions through porcine skin stabilized by polymeric emulsifier in µg/cm^2. 17-β-Estradiol (♦), progesterone (■), cyproterone acetate (▲) and finasteride (×). Indicated values are means (±S.D.) of three experiments.

ation rates were achieved with the exception of cyproterone acetate. This polymer caused the highest permeation rates for progesterone.

3.3.4. Microemulsions with silicon dioxide

A comparison of the skin diffusion of the hormones is shown in Fig. 6. As seen the following rank order of permeated hormones after 48 h from these formulations exists: finasteride > progesterone > cyproterone acetate > 17-β-estradiol. Silicon dioxide had a positive influence on the permeation of finasteride and 17-β-estradiol.

For clarification of the results a comparison of the fluxes (J) and the permeation coefficients (K_p) of all formulations are listed in Table 3.

As seen for all microemulsion systems containing 17-β-estradiol and progesterone higher permeation rates after 48 h of diffusion were obtained than from the polymeric coated liposomes (Biruss and Valenta, 2006). A higher skin permeation of finasteride than from the phospholipid liposomal system was

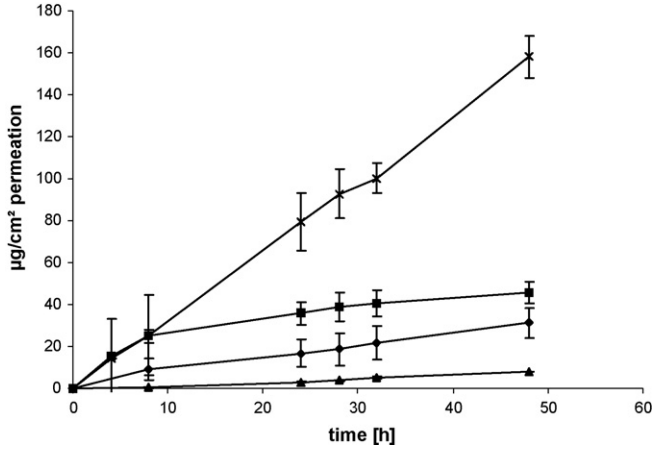

Fig. 5. Release profile of 17-β-estradiol, progesterone, cyproterone acetate and finasteride in microemulsions through porcine skin stabilized by polycarbophil in µg/cm^2. 17-β-Estradiol (♦), progesterone (■), cyproterone acetate (▲) and finasteride (×). Indicated values are means (±S.D.) of three experiments.

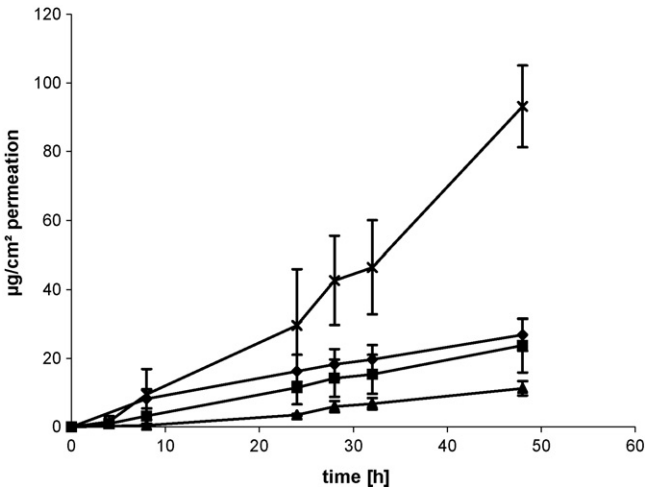

Fig. 6. Release profile of 17-β-estradiol, progesterone, cyproterone acetate and finasteride in microemulsions through porcine skin stabilized by silicon dioxide in µg/cm^2. 17-β-Estradiol (♦), progesterone (■), cyproterone acetate (▲) and finasteride (×). Indicated values are means (±S.D.) of three experiments.

achieved by using a polycarbophilic microemulsion system. With the exception of polycarbophilic microemulsion system higher diffusion rates of cyproterone acetate could be measured compared to the liposomal formulations.

3.4. Skin retention

The retention of the hormones in skin (Table 3) was proportional to the released hormones. The permeation rate was approximate 1.8-fold higher than the retention rate. The retention of 17-β-estradiol in skin was nearly in the same range as progesterone. Cyproterone acetate nearly exhibited the same retention as progesterone and 17-β-estradiol. In contrast finasteride obtained 1.9-fold higher results than the other investigated hormones with exception of polycarbophil.

3.5. Chemical stability

As seen in Table 4 all polymers effected a higher chemical stability for the used steroid hormones after an observation period of 6 weeks. However, a comparison of the chemical stability after 6 weeks of storage of cyproterone acetate between the pure microemulsions and the polycarbophilic gelified microemulsions does not show a significant difference. As seen an addition of polycarbophil increased the chemical stability of progesterone and finasteride. Polymeric emulsifier protected 17-β-estradiol against chemical degradation. Nearly 98% stability of cyproterone acetate could be received by the supply of silicone dioxide after a time period of 6 weeks.

As seen in Table 4 already after 6 weeks of storage about 41% of finasteride, 32% of progesterone, 27% of 17-β-estradiol and 13% of cyproterone acetate were degraded in the pure microemulsions.

As seen cyproterone acetate was the most stable hormone in all formulations followed by 17-β-estradiol, progesterone and finasteride. The stability of finasteride was acceptable

Table 4
Chemical stability of 17-β-estradiol, progesterone, cyproterone acetate and finasteride in % (w/w); $n = 3$

Week	17-β-Estradiol ± S.D.	Progesterone ± S.D.	Cyproterone acetate ± S.D.	Finasteride ± S.D.
Pure microemulsions				
0	100	100	100	100
1	99.48 ± 35.70	80.63 ± 34.44	97.01 ± 3.65	99.41 ± 14.57
2	92.59 ± 19.38	81.18 ± 19.92	97.21 ± 5.55	98.57 ± 20.51
3	92.50 ± 29.64	82.37 ± 8.44	97.15 ± 15.50	97.31 ± 12.90
5	91.84 ± 17.79	68.02 ± 5.49	87.90 ± 38.29	96.81 ± 71.65
6	73.05 ± 16.16	68.01 ± 11.71	87.24 ± 8.61	58.64 ± 1.43
Microemulsions with polycarbophil				
0	100	100	100	100
1	100.56 ± 12.74	98.94 ± 17.42	95.88 ± 8.46	111.36 ± 22.43
2	99.67 ± 6.50	98.65 ± 13.58	76.78 ± 3.12	111.10 ± 15.08
3	99.61 ± 4.50	98.81 ± 8.30	78.29 ± 2.07	105.12 ± 24.72
5	99.41 ± 7.76	82.82 ± 14.28	78.28 ± 4.91	73.18 ± 57.20
6	87.38 ± 6.72	82.87 ± 13.23	80.23 ± 17.03	72.10 ± 2.97
Microemulsions with silicon dioxide				
0	100	100	100	100
1	92.57 ± 6.40	91.25 ± 7.80	98.85 ± 9.74	102.05 ± 22.33
2	92.25 ± 19.45	90.66 ± 28.47	98.64 ± 7.10	101.74 ± 17.52
3	94.77 ± 13.60	92.86 ± 17.04	98.05 ± 14.16	101.62 ± 17.39
5	95.22 ± 9.14	72.49 ± 20.84	92.10 ± 20.15	72.41 ± 21.68
6	86.64 ± 4.50	71.01 ± 17.93	97.59 ± 3.07	68.39 ± 9.90
Microemulsions with polymeric emulsifier				
0	100	100	100	100
1	99.19 ± 23.42	91.37 ± 21.22	99.47 ± 10.01	100.24 ± 13.98
2	98.98 ± 14.06	90.09 ± 7.83	99.35 ± 33.25	100.10 ± 13.41
3	99.22 ± 13.46	90.30 ± 4.47	99.81 ± 21.36	100.29 ± 25.56
5	105.96 ± 7.20	82.82 ± 17.82	93.11 ± 3.13	86.98 ± 57.62
6	91.01 ± 2.14	81.11 ± 5.39	93.73 ± 20.96	66.04 ± 9.17

in all formulations until the fifth week. One reason for the degradation could be the higher hydrophilicity of the molecule.

Finally all additional polymers in microemulsions increased the chemical stability of the used hormones. These results are in good agreement with recently reported data dealing with polymers in liposomes (Biruss and Valenta, 2006). However, the chemical stability of cyproterone acetate was significantly higher than in the polymeric coated liposomes.

3.6. Rheological investigations

Viscosity measurement by oscillation is a gentle method without destruction. It provides information about the elastic properties G' (elastic modulus) and the viscous properties G'' (viscous modulus) of a preparation. Data of the formulations are presented in Table 3. G' is a measure for the recoverable energy stored elastically in the system, whereas G'' is a measure for the energy dissipated as viscous flow representing the real and imaginary parts of the complex dynamic shear modulus, respectively.

In order to get optimal applicable and skin tolerability formulations the final concentration of polycarbophil and polymeric emulsifier was 2% (w/w) and the final concentration of silicon dioxide was 6% (w/w). The measurement by oscillation of the pure microemulsions was under the detection limit. For this reason just gelified microemulsions were evaluated.

As can be seen in Table 5 silicon dioxide exhibited the highest G' and G'' values with predominant G' values at 1 Hz. It is obvious that silicon dioxide behaves completely different than the other investigated polymers. As indicated in Table 5 the elastic properties of siclicon-dioxide formulations were dominating at 1 Hz. In general, the polymeric emulsifier obtained higher viscosity results than polycarbophil. In order to get optimal applicable gels with a similar subjective skin feeling 6% of silicon dioxide and 2% of polycarbophil and polymeric emulsifier were used.

4. Discussion

Several reports are available dealing with microemulsions for topical and also for oral, parenteral, pulmonary and ocular use in order to achieve a better bioavailability of the incorporated drugs (Lawrence and Rees, 2000; Rhee et al., 2001; Lee et al., 2003). It is possible to solubilize a drug in microemulsion oil droplets that the drug solubility in the whole system is enhanced and the oil phase plays the role of a drug reservoir. The drug can diffuse through the dermis depending on the oil composition, the surfactant and on the cosurfactant by altering the structure of the stratum corneum (Rhee et al., 2001), on the other hand the lipophilic nature of the drugs and the nature of the skin may influence the permeation (Sentjurc et al., 1999).

NMR self diffusion coefficients provide a unique tool for the characterization of the microstructure of microemulsions

Table 5
Comparison of the elastic modulus (G') and the viscous modulus (G'') of in Pa at 1 Hz in polymeric stabilized formulations; $n = 3$

	Polycarbophil ± S.D.	Silicon dioxide ± S.D.	Polymeric emulsifier ± S.D.
17-β-Estradiol			
G'	2.14 ± 2.98	555.67 ± 17.01	13.27 ± 1.16
G''	9.58 ± 6.83	90.87 ± 10.86	15.30 ± 0.56
η'	1.52 ± 1.08	14.50 ± 1.75	2.43 ± 0.09
tan(δ)	10.80 ± 7.29	0.17 ± 0.03	1.15 ± 0.06
τ	28.50 ± 0.00	28.50 ± 0.00	28.50 ± 0.00
Progesterone			
G'	0.09 ± 0.01	1433.33 ± 270.25	15.80 ± 0.40
G''	2.70 ± 0.27	84.20 ± 12.45	17.33 ± 0.12
η'	0.33 ± 0.05	13.40 ± 2.00	2.76 ± 0.02
tan(δ)	22.23 ± 2.54	0.06 ± 0.00	1.10 ± 0.02
τ	28.50 ± 0.00	28.50 ± 0.00	28.50 ± 0.00
Cyproterone acetate			
G'	1.16 ± 1.60	2583.33 ± 90.18	41.33 ± 1.81
G''	7.79 ± 5.03	201.33 ± 21.03	28.73 ± 0.83
η'	1.24 ± 0.80	32.00 ± 3.30	4.57 ± 0.14
tan(δ)	15.33 ± 9.57	0.08 ± 0.01	0.70 ± 0.02
τ	28.50 ± 0.00	28.50 ± 0.00	28.50 ± 0.00
Finasteride			
G'	1.05 ± 0.88	1850.00 ± 210.00	8.88 ± 0.86
G''	9.23 ± 13.04	90.07 ± 21.30	13.23 ± 0.50
η'	1.47 ± 0.49	17.63 ± 7.37	2.11 ± 0.09
tan(δ)	11.42 ± 5.04	0.06 ± 0.02	1.50 ± 0.09
τ	28.50 ± 0.00	28.50 ± 0.00	28.50 ± 0.00
Placebo			
G'	0.63 ± 0.26	3646.67 ± 401.54	19.3 ± 6.64
G''	6.72 ± 1.42	133.67 ± 10.69	18.87 ± 3.79
η'	1.07 ± 0.23	21.27 ± 1.77	3.00 ± 0.60
tan(δ)	11.28 ± 2.38	0.04 ± 0.00	1.02 ± 0.19
τ	28.50 ± 0.00	28.50 ± 0.00	28.50 ± 0.00

(Lindman et al., 1999). In the case of the investigated eucalyptus oil/Brij-30/ethanol /water system the found normalized diffusion values are in the same order of magnitude for the oil and water components, as well as for the cosurfactant ethanol. Therefore a bicontinous structure is proposed for this multicomponent mixture. Another physicochemical characterisation of this microemulsion system was published recently, thereby using dynamic light scattering (DLS) measurements (Acharya et al., 2001) as one of the methods. Here they report a diffusion coefficient obtained from DLS experiments which is 1.7 orders of magnitude lower than the value we found for the micellar bound surfactant. But, as from a dynamics point of view, a bicontinuous microemulsion looks very much like a molecular solution, light scattering is unable to provide sufficient information about a structure (Langevin and Rouch, 1999), and for such systems the DLS derived diffusion coefficients are in general lower than those obtained by NMR (Caboi et al., 1997).

NMR self diffusion coefficients were also accessible for the steroid hormones despite their very low concentration. The interpretation of this value especially in terms of drug release finding a correlation to their transdermal flux is not an easy task. In the investigated pure microemulsion we can address some of the observed influences on the diffusion coefficients, like dependency on the molecular volume and viscosity, aggregation phenomena, specific interaction with the individual components of the vehicle, or solubility preferences in the polar or apolar phase. A clear separation and moreover quantitation of these different effects in such a multicomponent system cannot be achieved in detail. Attempts to correlate the drug release with NMR derived self diffusion coefficients have been published for Labrasol/Plurol isostearique/isostearylic isostearate/water microemulsions (Kreilgaard et al., 2000). Here the authors varied the composition of the carrier vehicle and studied the behaviour of a lipophilic and a hydrophilic model drug, respectively. They established a linear dependency with increased transdermal flux by higher mobility, though with a moderate correlation coefficient. In our investigation we studied very similar structured drugs in one microemulsion system. The measured self diffusion coefficients are different for the individual steroids.

From our results we can assume that the highest observed transdermal flux for finasteride is caused by an increased solubility in the apolar phase of the microemulsion, which can be deduced from a lower relative diffusion coefficient (D_{rel}, Table 2) for this drug in the NMR experiments. This may be one reason for the facilitate transport of finasteride. For the three other steroid hormones no correlation can be derived, as on the one hand the release rates are too similar and on the other hand the diffusion behaviour is also influenced by aggregation processes in case of 17-β-estradiol.

For the chemical stability of the steroids in the drug delivery system HPLC methods were used. The advantage of a chromatographic analytic method is the simultaneous determination of the main component and degradation products. Therefore, a permanent monitoring of degradation components is possible (Jianwei, 2002).

In earlier reports dealing with the degradation of 17-β-estradiol estrone was identified as main degradation product by the use of a certain HPLC method (Horace and Wotiz, 1962; Novakova et al., 2004). For all other investigated hormones certain methods were developed to monitor degradation products but with a lack of structure elucidation. These methods were used to identify the chemical stability of cyproterone acetate and finasteride in tablets (Segall et al., 2000, 2002; Syed and Amushumali, 2001). In contrast to semisolid formulations the steroid hormones exhibited high chemical stability over a long observation period.

A reason of chemical degradation of the selected drugs in the pure microemulsions might be chemical hydrolysis caused of the freely water. The polymer binds the water and interact with the droplets of the microemulsions (Huang et al., 1987). Additionally polyacrylic acid-derivates possess antimicrobial activity themselves. The mechanism of action may be explained by a high binding affinity to magnesium and calcium (Valenta et al., 1998). Beside a better antimicrobial stability might lead to a better chemical stability. Further studies concerning microbial stability have to be performed. The presence of ethanol and eucalyptus oil seem to have a synergistic influence on the chemical and microbial stability (Shahi et al., 2000). Consequently we did not use synthetic bacterial inhibitors.

Beside that eucalyptus oil containing large amounts of 1,8 cineol in combination with ethanol was reported to improve skin permeation in nifedipine containing microemulsions (Thacharodi and Panduranga, 1994) by perturbing the barrier function of the skin. On the other hand this fact may lead to skin irritation.

The innovation of our study is that both ingredients, eucalyptus oil as well as defined polymeric agents, should achieve a synergistic effect concerning the permeation rate. It is already reported that polymers are able to interact with surfactants on lipid bilayers and lead to an enhancement of the solubilization capacity of microemulsions (Sottmann, 2002). Referring to the investigated results it can be seen that certain polymers are able to enhance penetration of certain steroid hormones together with the other ingredients of the microemulsion system.

As drugs for the incorporation in microemulsions for topical application 17-β-estradiol, progesterone, cyproterone acetate and finasteride were chosen.

The utility of microemulsions as vehicles for transdermal delivery of the sexual hormone, 17-β-estradiol, was already studied and found to increase the permeability efficiently. In a previous study the influence of a synthetic polyacrylic acid derivate, Carbopol 940, and ethanol was separately compared. Referring to the results the polymer did not influence permeability while ethanol could improve the permeability (Peltola et al., 2003). In our study we investigated the influence of certain polyacrylic acid derivates and silicon dioxide combined with ethanol and eucalyptus oil and found a synergistic effect of all ingredients. 17-β-Estradiol is in use for the treatment of hormonal insufficiencies. Another indication of estrogens would be treatment of acne because of the inhibition of sebaceous gland activity (Downie et al., 2004).

Like 17-β-estradiol progesterone is in use to avoid postmenopausal side effects. Both drugs are often administered together to avoid endometrial carcinomas (Jasionowski and Jasionowski, 1977). Another study concluded that microemulsion systems improve the solubility of progesterone.

Although steroid hormones like 17-β-estradiol, progesterone (Nandi et al., 2003; Peltola et al., 2003) and cyproterone acetate (Kählig et al., 2005) have already been incorporated in microemulsions, finasteride has not been evaluated. In general, there are only few studies dealing with finasteride and topical delivery. Nevertheless the topically administered anti-androgen is interesting for the treatment of androgenetic alopezie (Mc Clellan and Markham, 1999). According to the convenient chemical and physical characteristic of finasteride like the chemical structure, the log *P* value, the lipophilicity properties and the saturation solubility, the permeation rates through skin are the highest in all preparations.

Recently (Biruss and Valenta, 2006) the influence of polymeric coated liposomes on skin permeation and on the chemical stability of some steroid hormones was investigated. It can be seen that the microemulsional formulations offered higher permeation rates for 17-β-estradiol and progesterone after 48 h of diffusion than the liposomal formulations. In case of chemical stability cyproterone acetate exhibited in the following study significant better stability properties than in the liposomes.

Additionally the skin retention amount of steroid hormones was evaluated for all formulations. The content is important concerning the mechanism of action of steroid hormones that involves the binding to specific intracellular receptors in certain areas of the skin (Whiting et al., 2000) beside the systemic efficacy.

It can be concluded that the addition of certain polymeric agents leads to an improvement of permeation of steroid hormones incorporated in microemulsions as a consequence of a synergistic effect between eucalyptus oil and polymers. All polymers can be recommended concerning stability because they are all able to improve chemical stability of the hormones in the vehicles with exception of polycarbophil and cyproterone acetate. As a next step the new semisolid formulations should be evaluated in extended in vivo studies on human skin and microbial stability studies should be carried out.

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