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Research paper

Skin permeation of different steroid hormones from polymeric coated liposomal formulations

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Abstract

In this study, the effect of various polymers (polycarbophil, chitosan–EDTA, polymeric emulsifier and carrageenan) on the permeation, the chemical and microbial stability of 17-β-estradiol, progesterone, cyproterone acetate (cpa) and finasteride incorporated in DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) liposomes has been investigated. The liposomes contained 1% (w/w) of the steroid hormones. Standard diffusion experiments were performed. Drug stability was monitored by analysing the steroid hormone content in the different formulations over a time period of 8 weeks and visually inspecting for microbial contamination. In addition, viscosity measurements were performed. The permeation rate could be improved by addition of polymeric agents depending on their type and drug. In all tested formulations, finasteride exhibited the highest diffusion. Both the chemical and the microbial stability of the hormones were significantly improved by the polymers in comparison to the pure liposomes after an observation period of 8 weeks. After that time microbial stability was still evident for all semisolid formulations. In contrast to this in the pure liposomes already after 2 weeks the steroid drugs showed complete insufficient chemical stability and microbial contamination. Additional rheological measurements indicated an influence of the polymers and drugs on the viscosity in all formulations. The elasticity predominated in nearly all polymeric formulations.

Keywords: 17-β-estradiol; Progesterone; cpa; Finasteride; Liposomes; Polymer; Stability; Diffusion experiments; Viscosity

1. Introduction

Over the past years, a large number of different steroid hormones have been used in topical formulations for various therapeutic purposes. In the last decade, liposomes containing 17-β-estradiol and progesterone are described [1–3] but few reports are dealing with cyproterone acetate (cpa) [4] or finasteride [5]. The transdermal drug delivery of hormones means a great benefit concerning the metabolism especially by avoiding the first pass effect [3]. Moreover, orally administered steroid hormones show poor bioavailability due to their hepatic metabolism [6]. That is the reason for high therapeutic doses causing negative systemic side effects [4]. The classically accepted mechanism of steroid hormone action involves the binding to specific intracellular receptors, initiating gene transcription and protein synthesis [7]. The presence of androgenic receptors in certain areas of the skin like sebaceous glands, hair follicle and keratinocytes are well known. Topically applied 17-β-estradiol is binding on its specific estradiol-α and -β receptors in skin. They are responsible for the typical efficacy of estradiol in brain, ovary, prostate and other tissues [8–10]. Although all investigated steroid hormones have a similar binding mechanism they have different functions and different lipophilic properties.

Numerous studies report that the application of liposomes on the skin surface is able to improve permeability for various entrapped drugs through the major barrier the stratum corneum [4,11–13]. One reason of the penetration enhancing effect of liposomes may be caused by the interactions between the intracellular lipids in the skin and the liposome bilayer. The extend of enhancement depends on the liposome size, lipid composition, the lipophilic nature of the drug and on the nature of the skin [14]. Liquid liposomes are applied directly to skin. Usually it is impossible to incorporate intact liposomes in creams because of interactions between the surface active surfactants and the liposomal layers [1].

Instead of creams polymers can be used to increase the viscosity of liposomal preparations. They are able to influence the drug penetration and stability by forming a stable layer

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around the vesicles in dependence on their concentration and type [15,16].

The aim of the present study was to incorporate 17-β-estradiol, progesterone, cpa and finasteride, respectively, in DPPC liposomes and to compare the skin permeation as well as the chemical stability of these hormones in the preparations under storage conditions. Chemical stability investigations were performed to exhibit that the stability of the drugs strongly depends on the used vehicle. In the past few years, the need for simple, selective analytical methods for the study of degradation products is grown. The use of certain HPLC methods supplies the determination of degradation products of the hormones [17]. The resulting liposomal formulations should be converted into semisolid preparations by the polymeric agents polycarbophil, chitosan–EDTA, polymeric emulsifier and carrageenan in order to investigate the influence of these polymers on the chemical stability of the hormones as well as the viscosity and elasticity of the resulting preparations compared to the pure liposomes.

2. Materials and methods

2.1. Materials

17-β-estradiol, progesterone, chitosan, EDTA and EDAC [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride] were purchased from Sigma (St Louis, USA). Cyproterone acetate (cpa) was a generous gift from Schering (Au). Finasteride was purchased from Kemprotec (UK). DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) was from Lipoid (Ge). Carrageenan (Satiagel CT 52) was a gift from Degussa (Ge). 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

Liposomes. Multilamellar vesicles were prepared by an already well-experienced hydration method [18] with DPPC by dissolving 17-β-estradiol, progesterone, cpa and finasteride at a final drug concentration of 1% (w/w) in a 3:1 chloroform/methanol solvent. In another flask, DPPC was dissolved in chloroform. Afterwards defined amounts of both solutions were mixed carefully and dehydrated by the use of a rotary evaporator until a thin homogenous lipid film was built inside the flask. The lipid films were re-hydrated with 60 °C of 10 mM phosphate buffer pH 7.2 in order to achieve a lipid content of 1 mg/ml by shaking for 15 min and annealing for 2 h.

Coated liposomes. All multilamellar liposomes containing the different drugs were coated by carrageenan, polycarbophil, polymeric emulsifier in a final concentration of (2% w/w) and chitosan–EDTA in a final concentration of (3% w/w) [19]. The polymers were added directly to the liposomes by gentle stirring. After a swelling time of about 2 h the resulting transparent semisolid preparations were stored in suitable vessels. Thus, the final concentration (1 mg/ml) of the lipids was equal for all investigated preparations.

Table 1
pH values of all liposomes and polymeric coated liposomes

Drug	Polymeric agent	pH value
17-β-estradiol	None	7.28
Progesterone	None	7.30
Cpa	None	7.30
Finasteride	None	7.29
17-β-estradiol	Polycarbophil 2%	3.92
Progesterone	Polycarbophil 2%	3.98
Cpa	Polycarbophil 2%	3.95
Finasteride	Polycarbophil 2%	3.90
17-β-estradiol	Polymeric emulsifier 2%	3.43
Progesterone	Polymeric emulsifier 2%	3.48
Cpa	Polymeric emulsifier 2%	3.44
Finasteride	Polymeric emulsifier 2%	3.42
17-β-estradiol	Carrageenan 2%	7.39
Progesterone	Carrageenan 2%	7.41
Cpa	Carrageenan 2%	7.40
Finasteride	Carrageenan 2%	7.35
17-β-estradiol	Chitosan–EDTA 3%	7.59
Progesterone	Chitosan–EDTA 3%	7.58
Cpa	Chitosan–EDTA 3%	7.58
Finasteride	Chitosan–EDTA 3%	7.61

For additional characterisation of the formulations measurements of the final pH value were performed and listed in Table 1.

2.2.1. Synthesis of the chitosan–EDTA conjugate

For the synthesis of chitosan–EDTA conjugate, the following already developed method was chosen [20]. Therefore, chitosan (1 g) was suspended in demineralised water (100 ml) and carefully dissolved by addition of HCl (1 M, 15 ml) until pH 3 was maintained. EDTA (3.63 g) was dissolved in this solution and sodium hydroxide solution (5 M, 3 ml) was added until pH 6 was received. To induce the formation of amide bonds between the amino groups of chitosan and the carboxyl groups of EDTA EDAC [1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride] was used at a final concentration of 0.1 M. The mixture was stirred by a magnetic bar overnight. For purification 2 days dialysis against demineralised water, 2 days against sodium hydroxide solution (5 mM) and 1 day against demineralised water was performed. The end product was received by lyophilisation and stored at −4 °C until use.

2.3. Solubility experiments

The solubility of all investigated hormones was analysed in the designated acceptor medium, which consists of propylene glycol/water (40 + 60 w/w). The propylene glycol was added in order to achieve sink conditions. An excess of each hormone was added to the media and stirred by a magnetic bar for 24 h at room temperature. After filtering (Minisart; Sartorius 0.45 μm) the drug content was analysed by HPLC. The experiments were performed in triplicate.

2.4. Skin preparation

Porcine abdominal skin which has a similar lipid composition to human skin [21] 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 1.2 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) and the permeation coefficients K_p (cm/h) of all formulations were calculated by the use of the concentration of the drug in the vehicle C_V (μg/cm^3) and compared.

$$J = K_p \times C_V$$

2.6. 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 a defined period. 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 for 8 weeks.

Therefore, a defined amount of formulation was dissolved in 1 ml methanol and centrifuged for 6 min. Twenty millilitre were analysed by HPLC. Like in other reports dealing with stability tests of steroid hormones [17,22] the comparison of the resulting chromatograms demonstrated that the main peak decreased and degradation products occurred within the observation period.

Before the investigation of each sample all preparations were visually observed for microbial contamination. In the case of evident contamination, the analysis was stopped.

2.7. 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).

2.7.1. Cyproterone acetate (cpa)

For the quantification of cpa a previously reported method was used [4]. The stationary phase was ODS-2, 5 μm column (150 mm × 3.6 mm) and the mobile phase consisted of acetonitrile/water (420 + 600 w/w). Detection wavelength was 280 nm. The retention time was approx. 7.7 min.

2.7.2. Progesterone

For the quantification of progesterone a previously reported method was used [2]. The stationary phase was Nucleosil 100 5 C-18 column (240 mm × 4.6 mm) and the mobile phase consisted of methanol/water (90 + 10 w/w). Detection wavelength was 240 nm. The retention time was approx. 4.2 min.

2.7.3. -β-estradiol

For the quantification of 17-β-estradiol the stationary phase was Nucleosil 100 5 C-18 column (240 mm × 4.6 mm) and the mobile phase consisted of methanol/water (70 + 30 w/w). Detection wavelength was 280 nm. The retention time was approx. 6.9 min.

2.7.4. Finasteride

For the quantification of finasteride a previously reported method was used [22]. The stationary phase was Nucleosil 100 5 C-18 column (240 mm × 4.6 mm) and the mobile phase consisted of methanol/water (70 + 30 w/w). Detection wavelength was 210 nm. The retention time was approx. 10.5 min.

Twenty millilitre of each sample were injected. To avoid any impurities pre-columns (40 mm × 3.6 mm) of the same material as the stationary phase were used.

For each drug, calibration curves were calculated on the basis of peak area measurements. They were generated with a correlation coefficient of 1.0 for finasteride, cpa, progesterone and a correlation coefficient of 0.9999 for 17-β-estradiol. The concentration range for finasteride was between 3.82 and 122.1 μg/ml, for cpa between 3.3 and 105.9 μg/ml, for progesterone between 7.73 and 123.7 μg/ml and for 17-β-estradiol between 44.1 and 705.5 μg/ml.

2.8. Rheological experiments

Oscillatory shear experiments were performed on a Haake rheometer Rotovisco RT 20 (Haake, Karlsruhe, Germany, thermo controller Haake F6/8). The rheometer tool was a thermostatically controlled cone/plate with 35 mm in diameter and 2° angle (C35/2Ti) for all polymeric coated formulations containing different concentrations of each polymer. During the experimental period, the plate temperature was maintained at 20 ± 1.5 °C. Sample amount was approx. 1 g. By this modus 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.

$$G' = G^* \cos(\delta)$$

$$G' = G^* \sin(\delta)$$

$$\eta\prime = G''/\omega$$

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.

2.8.1. Flow curves

To obtain flow curves additional experiments with the same formulations and tool (C35/2Ti) and the following parameters were performed: controlled rate (CR) modus, $\gamma = 1$–100; 100–$1\ s^{-1}$; 20 ± 1.5 °C. In brief, CR modus means that a controlled shear rate is applied. The exact viscosity values were calculated with the supply of reowin software 2.94 (Haake, Ge)
.

All rheological experiments were performed in triplicate.

2.9. Statistical data analysis

Results are expressed as the means of at least three experiments ± SD. Statistical data analysis was performed using the students *t*-test with $P < 0.05$ as a minimal level of significance.

3. Results

3.1. Liposomes

Multilamellar DPPC liposomes were prepared with incorporated steroid hormones by a well-experienced standard technique [18] at an average size between 200 and 300 nm. It is generally known that liposomal solutions are instable vehicles [16] for incorporated drugs The direct addition of carrageenan, polycarbophil, polymeric emulsifier and chitosan–EDTA serves to convert these solutions into transparent semisolid formulations and to improve the stability by forming a stable layer around the vesicles. The final drug content of all liposomal formulations was about 1% (w/w). In general, permeation rates and stability depend on the drug content.

3.2. Skin permeation

In order to obtain sink conditions for later permeation studies, the solubility in the acceptor medium was analysed and presented in Table 2 [4]. Permeation experiments were carried out with porcine skin, which has a similar lipid composition as human skin. Firstly, the diffusion of 17-β-estradiol, progesterone, cpa and finasteride incorporated in liposomes was compared (Fig. 1). Finasteride showed the highest significantly cumulative permeation rate after 48 h of diffusion. The amount was about 8-fold higher compared to the other tested hormones. The diffusion rates of 17-β-estradiol, progesterone and cpa were not significantly different and were in the same range. The rank order of the cumulative amounts permeated after 48 h of diffusion was finasteride > progesterone > 17-β-estradiol > cpa.

Table 2
Saturation solubility of 17-β-estradiol, progesterone, cpa and finasteride in mg/ml in propylene glycol/water (40 + 60 w/w); $n = 3$

Drug	Solubility ± SD
17-β-estradiol	0.072 ± 0.009
Progesterone	0.088 ± 0.015
Cpa	0.069 ± 0.001
Finasteride	1.955 ± 0.394

A similar situation (Fig. 2) was found when the liposomes were gelified by 2% (w/w) polycarbophil. The used polycarbophil consists of polyacrylic acid cross linked with divinylglycol. In general, the permeation results were lower than of the pure liposomes. A comparison of the cumulative amount of permeated hormones showed the same rank order as the diffusion from the pure liposomes.

Chitosan–EDTA was identified as interesting gelating agent for topical formulations [20]. Therefore, we tested this polymer in connection with the liposomes. The results are presented in Fig. 3. As clearly seen the rank order of the cumulative amount after 48 h is the same as in Figs. 1 and 2. The higher SD values resulted from the difficult standardisation of the natural occurring chitosan. The used polymeric emulsifiers are copolymers of acrylic acid modified by long chain (C10–C30) alkyl acrylates cross linked with allylpentaerythritol. In comparison to polycarbophil, this polymer is a more lipophilic matrix. Interestingly the permeation of cpa after 48 h of

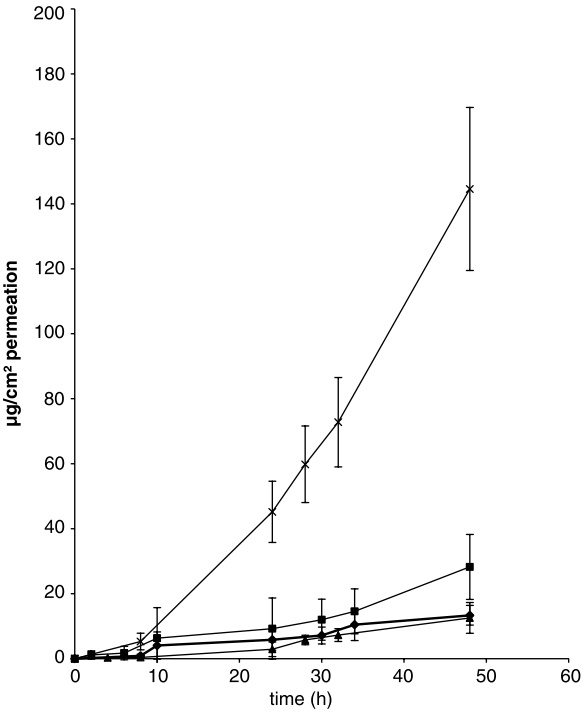

Fig. 1. Skin diffusion profile of 17-β-estradiol, progesterone, cpa and finasteride in liposomes through porcine skin in µg/cm^2. 17-β-estradiol (♦), progesterone (■), cpa (▲) and finasteride (×). Indicated values are means (±SD) of three experiments.

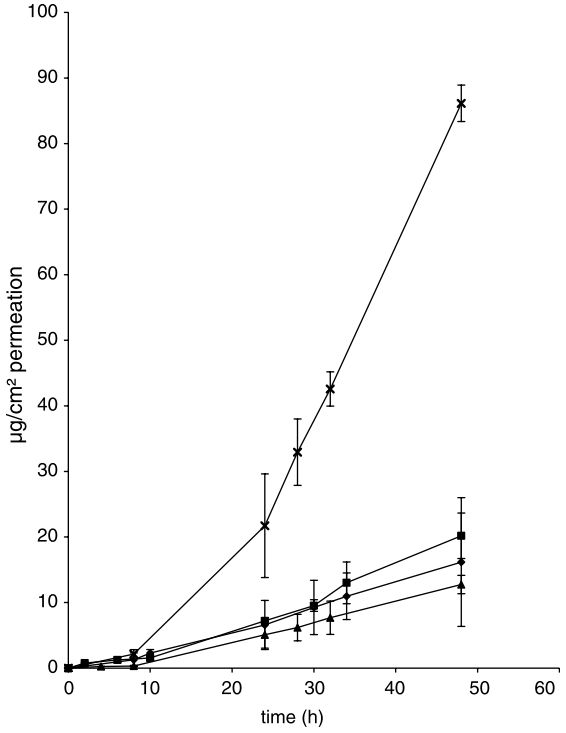

Fig. 2. Skin diffusion profile of 17-β-estradiol, progesterone, cpa and finasteride in liposomes through porcine skin stabilized by polycarbophil in µg/cm^2. 17-β-estradiol (♦), progesterone (■), cpa (▲) and finasteride (×). Indicated values are means (±SD) of three experiments.

liposomes coated by this polymer is higher than from the pure liposomes. The rank order of the cumulative permeation amount was different to the permeation rates described in Figs. 1–3, finasteride > cpa > progesterone > 17-β-estradiol.

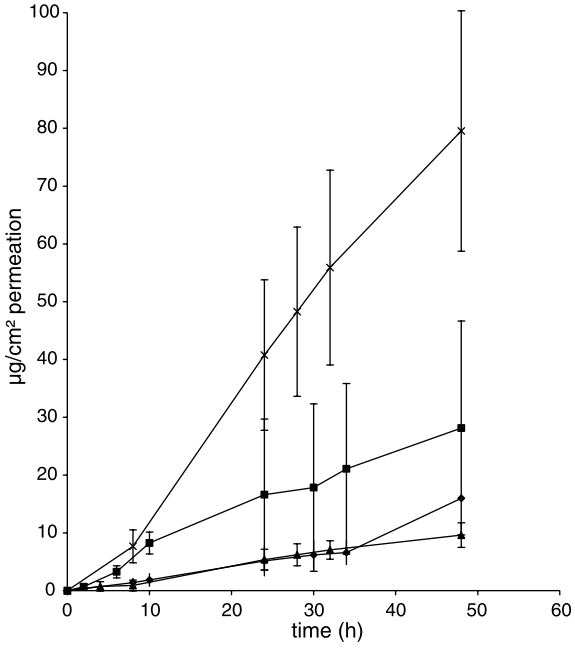

Fig. 3. Skin diffusion profile of 17-β-estradiol, progesterone, cpa and finasteride in liposomes through porcine skin stabilized by chitosan–EDTA in µg/cm^2. 17-β-estradiol (♦), progesterone (■), cpa (▲) and finasteride (×). Indicated values are means (±SD) of three experiments.

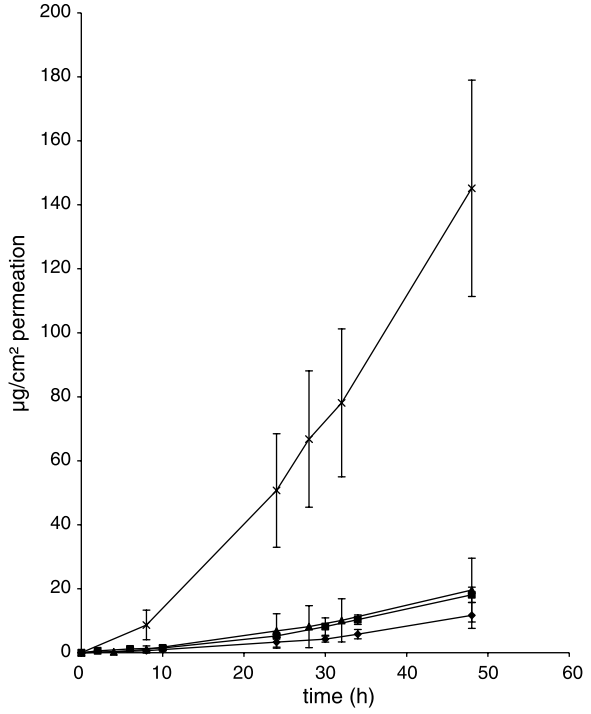

Fig. 4. Skin diffusion profile of 17-β-estradiol, progesterone, cpa and finasteride in liposomes through porcine skin stabilized by polymeric emulsifier in µg/cm^2. 17-β-estradiol (♦), progesterone (■), cpa (▲) and finasteride (×). Indicated values are means (±SD) of three experiments.

In Fig. 4, the following rank order after 48 h was given finasteride > cpa > progesterone > 17-β-estradiol. Finasteride exhibited the highest diffusion rate while the other drugs were at the same range.

It has to be pointed out that carrageenan increased the permeation rate at best with exception of 17-β-estradiol. In a previous study [23], carrageenan a polysaccharide prepared by alkaline extraction from red seaweed has been identified as permeation enhancer in topical preparations. In Fig. 5, it can be seen that the permeation rates of finasteride and progesterone were significant higher than from pure liposomes whereas the cumulative permeated amount of 17-β-estradiol and cpa were in the same range as from the pure liposomes. The following rank order after 48 h was given, finasteride > progesterone > cpa > 17-β-estradiol.

Comparing the cumulative amount (Figs. 1–5) after 48 h of permeation with the permeation coefficients and the fluxes (Table 3) it can be seen that the permeation of the drugs could be increased by addition of certain polymeric agents. Best results were obtained from carrageenan with exception of 17-β-estradiol. For this drug chitosan–EDTA but also polycarbophil showed a good permeation behaviour. In case of cpa polymeric emulsifier as well as carrageenan were able to improve its permeation. It can be observed that polycarbophil had just a very small permeation enhancing influence on cpa. An insignificant permeation enhancing behaviour can also be seen in the case of progesterone and chitosan–EDTA, respectively, finasteride and polymeric emulsifier.

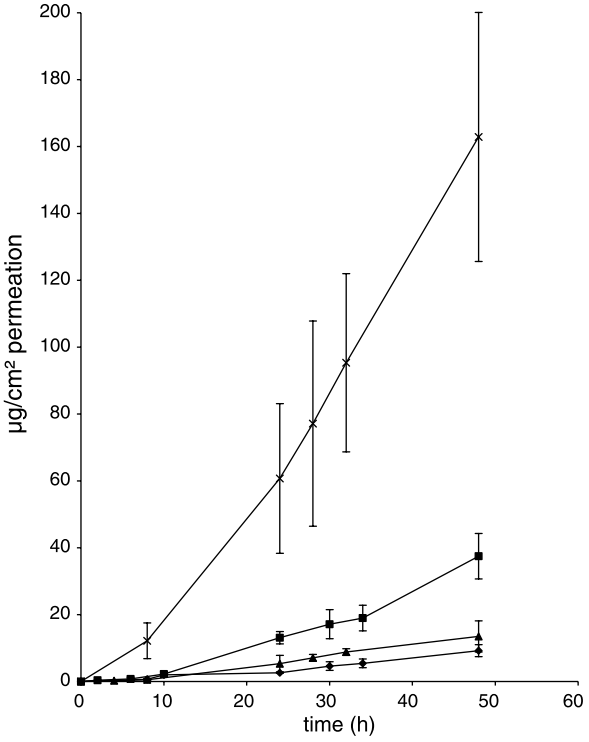

Fig. 5. Skin diffusion profile of 17-β-estradiol, progesterone, cpa and finasteride in liposomes through porcine skin stabilized by carrageenan in µg/cm^2. 17-β-estradiol (♦), progesterone (■), cpa (▲) and finasteride (×). Indicated values are means (±SD) of three experiments.

3.3. Chemical stability

As seen in Table 4, the hormones incorporated in liposomes exhibited very poor chemical stability under storage conditions. Already after two weeks of observation about 59% of finasteride and about more than 60% of 17-β-estradiol and progesterone and even 79% of cpa were degraded. Beside that a weekly visual observation of all formulations showed a strong microbial contamination of pure liposomes, whereas the coated liposomes gave no evidence of microbial contamination. As seen in Table 4, polymeric coated liposomes exhibited higher stability values within 8 weeks than the pure liposomes within 2 weeks. It can be seen that polycarbophil achieved best stability results for 17-β-estradiol and finasteride. With exception of carrageenan progesterone exhibited in all polymeric coated formulations the highest chemical stability. In comparison to cpa and finasteride, 17-β-estradiol is more stable after an observation period of 8 weeks. Furthermore, it can be noticed that finasteride shows the lowest chemical stability of all polymeric coated formulations. One reason may be the higher hydrophilicity regarding the chemical structure compared to the other hormones. Referring to the results it can be seen that drugs with a more hydrophilic structure are more likely to degrade in a higher extent. From the point of stability carrageenan was the polymer with the lowest stability properties with exception of cpa. All other polymers were able to exhibit higher stability of the hormones.

Table 3
Comparison of the fluxes (J) and permeation coefficients (K_p) of all formulations

	Polycarbophil	Chitosan–EDTA	Polymeric emulsifier	Carrageenan	Pure liposomes
17-β-estradiol					
J	0.3470	0.2996	0.2298	0.1836	O.2805
K_p	0.0058	0.0050	0.0038	0.0031	0.0047
Progesterone					
J	0.4096	0.5967	0.3534	0.7419	0.5203
K_p	0.0068	0.0099	0.0059	0.0124	0.0087
Cpa					
J	0.2730	0.2136	0.3981	0.2943	0.2602
K_p	0.0046	0.0036	0.0066	0.0049	0.0043
Finasteride					
J	1.7700	1.7379	3.0095	3.4048	2.9796
K_p	0.0295	0.0290	0.0502	0.0567	0.0497

Units: J = µg/cm^2/h; K_p = cm/h.

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

Week	17-β-estradiol ±SD	Progesterone ±SD	Cpa ±SD	Finasteride ±SD
Liposomes[a]				
0	100	100	100	100
1	96.25±36.08	94.15±39.18	114.00±12.58	99.28±6.59
2	38.99±14.94	38.95±6.39	21.33±3.76	41.39±4.59
Polycarbophil				
0	100	100	100	100
1	98.85±12.17	100±18.45	94.58±7.19	60.10±2.67
2	95.78±37.98	118.26±36.36	80.50±13.44	63.68±12.44
6	88.30±23.91	100.73±13.02	74.45±12.19	63.43±11.37
8	87.37±31.27	98.75±6.38	66.60±15.18	59.95±10.83
Chitosan–EDTA				
0	100	100	100	100
1	97.38±22.56	92.18±8.27	98.66±24.84	84.72±14.84
2	101.13±23.94	109.37±19.47	98.34±23.01	73.55±16.23
6	85.19±10.90	98.60±28.70	77.35±14.15	58.70±23.00
8	82.51±12.18	97.24±4.64	75.32±20.06	57.28±4.48
Polymeric emulsifier				
0	100	100	100	100
1	95.11±5.36	99.75±15.11	96.57±14.53	98.62±22.33
2	93.87±13.36	119.81±15.11	89.86±16.58	70.71±6.50
6	74.37±18.01	114.31±34.31	69.82±24.38	55.63±10.60
8	73.94±31.32	115.57±12.01	67.25±10.54	54.21±16.53
Carrageenan				
0	100	100	100	100
1	99.36±2.03	98.87±57.29	98.66±24.84	74.27±13.52
2	99.10±24.76	111.80±13.32	98.34±23.01	74.18±47.40
6	73.15±9.29	107.39±6.27	95.12±16.28	57.02±11.70
8	71.18±13.07	57.79±3.57	93.29±32.22	56.36±22.75

[a] The drug analysis was terminated to 2 weeks because of microbial massive spoilage.

3.4. Rheological investigations

In order to characterize all investigated semisolid formulations and to receive a better comparison between them rheological experiments have to be performed. First of all flow curves (data not shown) were performed. All curves exhibited pseudoplastic flow character. There progesterone and 17-β-estradiol increased the viscosity in contrast to cpa, finasteride

Table 5
Comparison of the elastic modulus (G') and the viscous modulus (G'') of in Pascal at 10 Hz in polymeric stabilized formulations; $n=3$

	Polycarbophil ± SD	Chitosan–EDTA ± SD	Polymeric emulsifier ± SD	Carrageenan ± SD
17-β-estradiol				
G'	88.6 ± 7.18	2533.33 ± 181.48	119 ± 2.65	71.87 ± 4.00
G''	55.5 ± 1.47	1203.33 ± 28.87	83.03 ± 1.08	36.53 ± 1.88
η'	0.87 ± 0.02	18.73 ± 0.46	1.29 ± 0.02	0.57 ± 0.03
Progesterone				
G'	79.20 ± 0.20	3280 ± 186.82	70.17 ± 1.14	70.2 ± 2.65
G''	52.23 ± 0.84	751.67 ± 32.72	61.73 ± 1.3	41.13 ± 0.42
η'	0.81 ± 0.02	11.67 ± 0.49	0.96 ± 0.02	0.64 ± 0.01
Cpa				
G'	69.9 ± 0.96	2080 ± 183.3	130.33 ± 5.51	43.4 ± 6.27
G''	52.73 ± 0.91	1306.67 ± 35.12	99.4 ± 2.96	33.2 ± 2.25
η'	0.82 ± 0.02	20.33 ± 0.50	1.55 ± 0.04	0.52 ± 0.04
Finasteride				
G'	70.34 ± 0.4	449 ± 99.14	64.83 ± 0.61	55.8 ± 1.68
G''	50.73 ± 1.07	852.67 ± 99	52.33 ± 0.65	35.37 ± 0.64
η'	0.79 ± 0.02	13.27 ± 1.55	0.81 ± 0.01	
Control				
G'	38.43 ± 1.02	194.67 ± 56.71	41.87 ± 0.8	14.2 ± 3.02
G''	33.57 ± 0.15	753 ± 140.68	41.47 ± 0.9	25.97 ± 2.61
η'	0.52 ± 0.00	11.72 ± 2.18	0.65 ± 0.01	0.41 ± 0.04

or even the placebo. The reason of this rank order can be found in the different particle size of the hormones and the different ability of the polymers to dissolve the particles.

In contrast to conventional flow curves, 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 5. 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 formulations, the final concentration of polycarbophil, polymeric emulsifier, carrageenan was 2% (w/w) and the final concentration of chitosan–EDTA was 3% (w/w).

As shown in Table 5, the highest value of G' and G'' was measured for chitosan–EDTA. In all other cases, G' and G'' exhibited elastic and viscous properties but elastic properties are predominant with exception of control-chitosan–EDTA, control-carrageenan and finasteride-chitosan–EDTA. Nearly all preparations showed a higher G' than G'' at 10 Hz.

4. Discussion

Many reports are available dealing with incorporated drugs in liposomes not only for topical use but also for parenterale and pulmonal use in order to achieve a better bioavailability [4,13,24]. As mentioned before the extend of the penetration enhancing effect of liposomes depends on the liposomes size, the lipid composition, the lipophilic nature of the drugs and the nature of the skin [14]. To receive high cumulative permeation amounts on the one hand large multilamellar DPPC liposomes incorporated with lipophilic steroid hormones were chosen. On the other hand, these liposomes were coated by different polymers that are able to act as enhancers by improving the solubility of the drugs [25].

A major disadvantage of liposomes is their microbial and chemical instability [15,26]. Referring to our stability results, it could be shown that the chemical stability of the drugs strongly depends on the stability properties of the used vehicle. The extend of hormone degradation depends on pH value, temperature and water content in the formulations. The results clearly show that the pure liposomes with the highest water content show poor stability whereas the polymeric coated formulations with less water content are much more stable. Concerning to the temperature it has to be pointed out that we stored all formulations at room temperature under the same condition. The aim was to study the stability of all formulations under storage conditions. Within an observation period of 8 weeks we can conclude that the different pH values of the formulations (Table 1) have just a little influence concerning the stability. Therefore, a longer observation period in following studies should be chosen. 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 [27]. One strategy to increase the chemical and microbial stability of drugs in liposomes is the addition of defined polymers [3,15] by forming a stable layer around the vesicles. In one study, carboxymethylcellulose was found to exhibit stabilizing properties for an instable model drug in DPPC liposomes [15].

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 [28,29]. 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 cpa and finasteride in tablets [17,22,30]. In contrast to semisolid formulations, the steroid hormones exhibited high chemical stability over a long observation period.

Another fact is the anti-microbial activity of polymers themselves like in case of polyacrylic acid-derivates and carrageenan. The mechanism of action may be explained by a high binding affinity to magnesium and calcium [20]. These bivalent cations are essential components in the outer membrane of bacteria. This binding affinity is higher for chitosan–EDTA. As interesting drug candidates for incorporation in liposomes for topical application 17-β-estradiol, progesterone, cpa and finasteride were chosen. Whereas for 17-β-estradiol topical formulations like Linoladiol®, Ovestin® and for progesterone products like Progestogel® are available no products with cpa or finasteride are on the market.

The sexual hormone 17-β-estradiol is in use for the treatment of postmenopausal syndrome and preventing osteoporosis and physiological side effects of the menopause. Another indication of estrogens would be treatment of acne because of the inhibition of sebaceous gland activity [31–33]. However, reduction of gland size and sebum secretion in humans has only be demonstrated using large doses of estrogens with side effects [34]. Therefore, topical preparations, which assure a sufficient level at the sebaceous glands could offer a local acne treatment with decreased side effects [31].

Rat skin permeation of 17-β-estradiol was compared from pure liposomes with liposomal gels coated by polyacrylic acid and hydroxypropylcellulose and found to be improved by the polymers [3]. Other studies show a correlation between type of liposomes e.g. unilamellar or multilamellar and skin diffusion rates of 17-β-estradiol which were higher from multilamellar liposomes [13,24]. Although it was found that 17-β-estradiol increased the lipid mobility and progesterone decreased the fluidity of liposomes [7] the addition of progesterone resulted in higher permeation rates than liposomes with 17-β-estradiol alone.

Like 17-β-estradiol progesterone is in use to avoid postmenopausal side effects. Both drugs should be administered together to avoid endometrial carcinomas. Topical progesterone seems to be useful for the therapy of chronic vulvar dystrophy [35] and for reducing the symptoms of peripheral vascular disease. Recent research showed that salivary, but not serum or urinary levels of progesterone were elevated after topical application of progesterone cream to pre- and postmenopausal women which could help prevent systemic side effects [36]. Another indication of topical administered progesterone in high doses is the treatment of acne by affecting the sebaceous glands [34]. An interesting fact is that progesterone receptors were also found in human cornea, which is useful for the treatment of keratoconjunctivitis sicca for postmenopausal women [37]. Penetration enhancers like laurocapram, phloretin and ketocholestanol were used to increase the skin permeation of progesterone [2,38] and found to be suitable but the skin compatibility is insufficient. Therefore, liposomes usually non-irritating should be preferred.

Although topical cpa formulations offering sufficient concentration on the target side the sebaceous glands, but with reduced systemic absorption would be desirable for the treatment of acne no product is available. In one clinical study, a liposomal formulation was tested and found to improve acne lesions significantly [39]. The mechanism of action is believed to result in a local suppression of sebaceous secretion [40]. The results of the release of cpa in DPPC liposomes are also corresponding to a previous study in which DPPC liposomes were generally classified as convenient vehicles obtaining best diffusion rates depending on their lipid content [4].

Recently it has been demonstrated that a cubic gel is another possible carrier for cpa with high permeation rates [41] and an excellent chemical stability of cpa.

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. Products like Proscar® tablets are on the market but similar to cpa no topical products are available. Finasteride inhibits the 5α-redutase type 1, located in the sebaceous glands, less than type 2, located in the hair follicle [42]. These isoenzymes convert testosterone to dihydrotestosterone which shortens the hair follicle and miniaturizes scalp follicles [5,43,44]. A topical formulation can be applied on the condition that finasteride is transported to the sebaceous glands. Liposomes have a good chance to fulfil this demand. On one hand it was shown that the topical administration of finasteride caused local inhibition of sebaceous gland growth in hamsters [45,46] and on the other hand finasteride exerted severe systemic side effects [45].

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.

Although it could be supposed that steroid hormones from liposomal solutions can permeate skin more easily the opposite was proven in experiments [47]. An explanation might be the fact that elastic vesicles are most efficient under non-occlusive conditions as Cevc and Blume found out [48].

It can be concluded that the permeation of steroid hormones incorporated in liposomes can be increased by addition of certain polymeric agents. All polymers can be recommended because they are all able to improve chemical and microbial stability of the hormones in the liposomes. The viscosity of the polymer coated liposomes strongly depends on the used hormones. It is the question whether the polymers are able to improve solubility of the used drugs by a sort of supersaturation too [25,49] chitosan–EDTA is a promising vehicle especially on skin because of its broad spectrum against bacteria and the permeating enhancing properties by disrupting the intercellular tight junctions [50]. As a next step, the new semisolid transparent formulations should be evaluated in extended in vivo studies on human skin.

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