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# Temoporfin-loaded liposomal gels: Viscoelastic properties and *in vitro* skin penetration


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ABSTRACT

Temoporfin (mTHPC) is a potent second-generation photosensitizer. The primary object of this study was to develop a topical mTHPC-loaded liposomal hydrogel able to deliver mTHPC into the stratum corneum (SC) and deeper skin layers. This study was conducted (1) to determine the effect of carbomer concentration, used as a gelling agent, and the effect of phosphatidylcholine (PC) content of lecithin, used for the liposome preparation, on viscoelastic properties and viscosity of liposomal gels and (2) to determine the relationship between rheological properties of gels and the skin penetration of mTHPC. Liposomal hydrogels revealed plastic flow behaviour. The increase of carbomer concentration induced a domination of elastic over viscous behaviour of gels. There was an inverse relationship between the elasticity of gels and mTHPC-penetration. Viscosity also increased with the increment of carbomer concentration, reducing the mTHPC-penetration. Liposomal gels containing lecithin of smaller PC-content (i.e. smaller purity) exhibited a more elastic solid behaviour than gels containing lecithin with high PC-content, and showed smaller mTHPC-penetration. The gel containing 0.75%, w/w, carbomer and lecithin with high PC-content was considered to be the optimal formulation, since it delivered high amounts of mTHPC to the SC and deeper skin layers, and it possessed desirable rheological properties.


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## 1. Introduction

Temoporfin (mTHPC) is a very potent second-generation synthetic photosensitizer, activated at 652 nm wavelength, which allows a laser light induction of at least 1 cm body penetration, high tumor selectivity and residual photosensitivity of only two weeks (Ris et al., 1993; Hopper, 2000; Biel, 2002). It has been shown to be effective in the photodynamic therapy (PDT) of primary non-melanomatous tumors of the skin of the head and neck after its intravenous administration (Kübler et al., 1999).

There is a great interest in the development of topical dosage forms containing mTHPC, which could be used in the topical PDT of psoriasis and superficial skin cancer lesions. Topical dosage forms would simplify the therapy, since the skin is readily accessible for topical treatment, increase the drug concentration in the skin, enhance patient compliance and restrict the residual photosensitivity only to the site of application. However, mTHPC is a highly hydrophobic drug with a large molecular weight of 680 Da, which makes its percutaneous penetration very low.

In recent years liposomes have been intensively studied as drug carrier systems for topical delivery, since they have the potential to enhance drug penetration into the skin (El Maghraby et al., 2001; Verma et al., 2003a; Betz et al., 2005; Dragicevic-Curic et al., 2008), improve therapeutic effectiveness (Seth et al., 2004; Mura et al., 2007), decrease side-effects (Seth et al., 2004) and act as a local depot for sustained release of dermally active components (Schreier and Bouwstra, 1994). Several mechanisms of vesicle-skin interactions have been described in the literature. The theory that intact vesicles could penetrate into and even through stratum corneum (SC), acting as drug carrier systems (Cevc and Blume, 1992) is not supported by other authors, who found no evidence of intact vesicles in the deeper skin layers (Lasch et al., 1991; Zellmer et al., 1995; van Kuijk-Meuwissen et al., 1998). It was suggested that most likely vesicles disintegrate at the skin surface and that their components penetrate molecularly dispersed into the intercellular lipid matrix, where they mix with the SC lipids modifying the lipid lamellae (Hofland et al., 1994; Zellmer et al., 1995; Kirjavainen et


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al., 1996) and inducing new vesicle-like structures (Hofland et al., 1994). These conformational changes of SC lipids are strongly correlated with the enhanced percutaneous drug penetration (Coderch et al., 1999). Regarding enhanced drug penetration, fluid liquid-state vesicles composed of non-hydrogenated phospholipids have been proven to be superior to rigid gel-state vesicles composed of hydrogenated phospholipids (El Maghraby et al., 2001; Verma et al., 2003a; Seth et al., 2004).

Due to the aforementioned advantages, in this study liquid-state liposomes were chosen to serve as the drug delivery system for mTHPC. Since topically applied liposomes may leak from the application site, they were mixed with carbomer hydrogels in order to obtain semisolid liposomal formulations. In addition, liposomal gels were found to enhance the skin retention of drugs, i.e. they provide higher and sustained skin concentrations of drugs compared to conventional gels and creams, without enhancing the systemic absorption of drugs (Kim et al., 1998; Seth et al., 2004; Fresno Contreras et al., 2005; Padamwar and Pokharkar, 2006). Therefore, in the case of drugs that should act topically, liposome-based formulations are known to provide a localized and controlled drug delivery, acting as a drug reservoir for continuous drug delivery (Singh and Vyas, 1996; Kim et al., 1998; Glavas-Dodov et al., 2002; Puglia et al., 2004). Some authors suggest that liposomal phospholipids may mix with the intercellular lipids and cause a swelling effect. These swollen lipids subsequently result in the formation of an intracutaneous drug depot (du Plessis et al., 1994; Puglia et al., 2004).

Rheological properties, i.e. flow and viscoelastic properties, of gels play an important role in the mixing and flow characteristics of materials, their packaging into containers (Schott, 2000), physical stability (Tamburic and Craig, 1996) and patients acceptability (Wang et al., 1999). In addition, rheological properties have influence on drug release from semisolid formulations (Bonacucina et al., 2004; A-sasutjarit et al., 2005), its subsequent penetration into the skin, and on adhesive properties (Tamburic and Craig, 1996; Bonacucina et al., 2004), i.e. the required contact time between the treated area and the gel.

The main aim of this study was to develop a mTHPC-loaded liposomal hydrogel which would be able to deliver the photosensitizer in an efficient dose into the SC and deeper skin layers. In order to achieve that, liposomal gels of different carbomer concentration and phosphatidylcholine (PC) purity were prepared, analyzed for their rheological properties and afterwards investigated for their ability to deliver mTHPC *in vitro* into human skin. The goal of the study was (1) to investigate the effects of carbomer concentration and different phospholipids, (i.e. different purity of PC) in liposomal gels on the viscoelastic and flow properties of liposomal hydrogels and (2) to determine the relationship between rheological properties of liposomal gels and the skin penetration of mTHPC from gels. This study presents the first study investigating the penetration of mTHPC from semisolid formulations into the skin.

## 2. Materials and methods

### 2.1. Materials

Soybean lecithins Lipoid S-100 and Lipoid S-75 were a gift from Lipoid GmbH (Germany). According to the manufacturers certificates Lipoid S-100 contained 96.2% PC, while Lipoid S-75 contained 67.5% PC. 7,8-dihydro-5,10,15,20-tetrakis-(3-hydroxyphenyl) porphyrin (temoporfin, mTHPC) was a gift from biolitec AG (Germany). Carbomer (Carbopol 980 NF) was purchased from BFGoodrich (USA), propylene glycol from BASF (Germany) and bovine serum albumine (BSA) from Sigma–Aldrich (Germany). The preservatives: methylparaben, ethylparaben, propylparaben, butylparaben in phenoxyethanol (Sepicide HB) and imidazolidinyl urea (Sepicide SI) were a gift from Seppic (France). All other chemicals were of analytical grade.

### 2.2. Preparation of liposome dispersions

The composition of liposome dispersions containing the phospholipid Lipoid S-75 (LDS-75) and the phospholipid Lipoid S-100 (LDS-100) are represented in Table 1. The liposome dispersions were prepared by the conventional film method. mTHPC was dissolved in the methanol:chloroform (2:1, v/v) solution of phospholipids (Lipoid S-75 or Lipoid S-100). These mixtures were dried to a thin film at 50 °C by slowly reducing the pressure using a rotary evaporator (BÜCHI Vacobox B-177, BÜCHI, Switzerland). The obtained films were kept under vacuum (1 mbar) for 2 h at room temperature, flushed with nitrogen, and then hydrated with the appropriate amount of phosphate buffer saline (PBS) pH 7.4 for 30 min at room temperature. After that, liposome dispersions were extruded through polycarbonate membranes of different pore sizes (400 nm, 200 nm, 100 nm and 50 nm) with the help of the Emulsiflex C5. The pH values of obtained liposome dispersions were determined directly in the samples at room temperature (HI 8417, Hanna Instruments, USA).

For penetration studies liposome dispersions were diluted with PBS pH 7.4 in order to obtain dispersions with the same phospholipid and mTHPC-content as they were in the liposomal gels (10%, w/w, phospholipids and 0.15, w/w, mTHPC).

### 2.3. Visualization of liposomes in dispersions by cryo-electron microscopy

Liposome dispersions were diluted with PBS pH 7.4 and 5 μl of diluted dispersions were put onto a perforated coated net of copper (Quantifoil R 1.2/1.3, 400 mesh). Excess of samples was removed with a sheet of filter paper. The samples were quickly frozen with liquid ethane (−170 °C to −180 °C) in a cryo-box (Carl Zeiss NTS GmbH). Excess ethane was removed by blotting the samples in the cold and the samples were placed with the help of a cryo-transfer device (Gatan 626 Single Tilt Cryotransfer System)

**Table 1**
Composition of the different liposome dispersions and liposomal hydrogels.

| Sample | mTHPC (%, w/w) | S-75 (%, w/w) | S-100 (%, w/w) | Carbopol 980 (%, w/w) | NaOH 18%sol. (%, w/w) | Sepicide HB (%, w/w) | Sepicide CI (%, w/w) | Propylene Glycol (%, w/w) | EDTA (%, w/w) | Water phase[a] (%, w/w) |
|---|---|---|---|---|---|---|---|---|---|---|
| LDS-75 | 0.225 | 15 | – | – | – | – | – | – | – | ad 100 |
| LG1S-75 | 0.150 | 10 | – | 0.50 | 1.15 | 0.1 | 0.07 | 5 | 0.05 | ad 100 |
| LG2S-75 | 0.150 | 10 | – | 0.75 | 1.72 | 0.1 | 0.07 | 5 | 0.05 | ad 100 |
| LG3S-75 | 0.150 | 10 | – | 1.00 | 2.30 | 0.1 | 0.07 | 5 | 0.05 | ad 100 |
| LDS-100 | 0.225 | – | 15 | – | – | – | – | – | – | ad 100 |
| LG1S-100 | 0.150 | – | 10 | 0.50 | 1.15 | 0.1 | 0.07 | 5 | 0.05 | ad 100 |
| LG2S-100 | 0.150 | – | 10 | 0.75 | 1.72 | 0.1 | 0.07 | 5 | 0.05 | ad 100 |
| LG3S-100 | 0.150 | – | 10 | 1.00 | 2.30 | 0.1 | 0.07 | 5 | 0.05 | ad 100 |

[a] In the case of liposome dispersions LDS-75 and LDS-100 PBS pH 7.4 was used as the water phase, while for all liposomal gels purified water was used.

in a pre-cooled transmission-cryo-electron microscope (Philips CM 120). Microscopy was performed at 120 kV.

### 2.4. Preparation of liposomal hydrogels

The concentration of carbomer in the hydrogels was 1.5%, 2.25% and 3%, w/w. Hydrogels of three different polymer concentrations were prepared by the following procedure: carbomer resin was dispersed in purified water in which propylene glycol (15%, w/w), edetate disodium (0.15%, w/w), and the preservatives (Sepicide HB 0.30%, w/w, and Sepicide CI 0.21%, w/w), were previously added and left to stay for 24 h. The mixture was stirred the next day (500 rpm/min, 15 min) until the carbomer resin was homogeneously dispersed and then neutralized by addition of an accurate amount of 18%, w/w, solution of sodium-hydroxide (solution of sodium-hydroxide:carbomer = 2.3:1, w/w).

In order to obtain liposomal gels, liposome dispersions LDS-75 and LDS-100 were incorporated into previously prepared carbomer hydrogels in the ratio 2:1, w/w, by mixing them into the gel by an electrical mixer at 200 rpm/min for 5 min (Heidolph RZR 2020, Germany). The concentration of phospholipids in obtained liposomal gels was 10%, w/w, the concentration of mTHPC was 0.15%, w/w, and the concentration of carbomer was 0.5%, 0.75% or 1.0%, w/w, dependent on the carbomer concentration in the hydrogel (1.5%, 2.25% or 3%, w/w) used for the incorporation of liposomes. Liposomal gels containing the liposome dispersion LDS-75 and 0.5%, 0.75% or 1.0%, w/w, carbomer were labelled as LG1S-75, LG2S-75 and LG3S-75, respectively. Gels containing the liposome dispersion LDS-100 were labelled in the same manner as LG1S-100, LG2S-100 and LG3S-100. The composition of final liposomal hydrogels is represented in Table 1. The pH values of gels were determined directly in the samples (HI 8417, Hanna Instruments, USA).

### 2.5. Physical characterization of liposomes in dispersions and hydrogels by photon correlation spectroscopy (PCS)

The particle size (*z*-average) and polydispersity index (PDI) of liposomes in dispersions and hydrogels were determined by PCS using the Zetasizer Nano ZS (Malvern Instruments, Herrenberg, Germany). Liposome dispersions were diluted with PBS pH 7.4 prior to the measurements. For the particle size measurements 5 μl of the vesicle dispersions were diluted with 495 μl of PBS (pH 7.4). To obtain the particle size and PDI of liposomes incorporated in the hydrogels, 0.2 g of the hydrogels was diluted (1:10, w/w), with PBS 7.4, mixed until a slightly opaque dispersion was obtained and afterwards the dispersion was centrifuged at 3000 rpm. 50 μl of the supernatant was further diluted with 450 μl of PBS (pH 7.4) and analyzed for the *z*-average and PDI of liposomes. All measurements were performed at 25 °C after 5 min of thermal equilibration. The Zetasizer Nano ZS detects backscattering at an angle of 173° for an improved sizing of especially larger particles at higher concentrations. The particle size was calculated with the “Dispersion Technology Software 3.30” and the analysis function “general purpose” that bases on a CONTIN algorithm. A spherical form of particles, a viscosity of the medium of 0.89 mPa s and refractory index of 1.33 were assumed.

### 2.6. Rheological evaluation of liposomal hydrogels

Rheological properties of liposomal hydrogels were performed by using the rotational and oscillatory rheometer (Rheolab MC 120, Paar Physica, Germany).

Continuous flow tests of 48 h old liposomal hydrogels were performed at 20 ± 0.1 °C by using the cone/plate-measuring device MK 22 (radius of measuring cone 25 nm, angle of measuring cone 1°). The measurements were carried out by increasing the shear rate from 0 $s^{-1}$ to 200 $s^{-1}$ and decreasing it back to 0 $s^{-1}$, each stage lasting 200 s.

Viscoelastic materials exhibit viscous flow combined with elastic deformation when stressed (Boulmedarat et al., 2003). Viscoelastic behaviour of gels was studied using dynamic (oscillatory) measurements. Firstly, the linear viscoelastic area was determined, wherein viscoelastic parameters are independent of strain amplitude. This strain sweep test was carried out at a fixed frequency of 1 Hz and the initial and final strains were set at 0.6% and 100%, respectively. Once this region was established frequency sweep tests were performed at a constant strain of 1.0%, within the determined linear viscoelastic regime, and frequency range from 0.1 Hz to 10 Hz. The measurements were performed at 20 ± 0.1 °C by using the cone/plate-measuring device MK 24 (radius of measuring cone 75 nm, angle of measuring cone 1°).

### 2.7. In vitro skin penetration studies

#### 2.7.1. Skin preparation

Human abdominal skin obtained after plastic surgery was used for the penetration studies. The subcutaneous fatty tissue was completely removed from the skin using a scalpel and surgical scissors. After that the skin surface was cleaned with Ringers' solution and allowed to dry. The skin was packed in aluminum foil and stored in a polyethylene bag at −20 °C. Under these conditions the skin is, with respect to the penetration studies, stable for 3–6 months (Harrison et al., 1984). Full-thickness skin prepared in the aforementioned way was used for the *in vitro* skin penetration studies, which have been carried out under the approval of the Ethics Committee of the Friedrich-Schiller University Jena.

#### 2.7.2. Preparation of Franz diffusion cells

The nominal surface of the Franz cells was 3.14 $cm^2$ and acceptor compartments had a capacity of approximately 13 ml. Skin disks of 36 mm were punched out, allowed to thaw, cleaned with Ringers' solution and transferred onto the Franz diffusion cells. The acceptor compartment contained PBS pH 7.4 with 1%, w/w, BSA in order to maintain sink conditions. The prepared Franz cells were stored in a refrigerator (4 °C) overnight in order to hydrate the skin. The next day, magnetic bars were placed into the Franz cells. The temperature of the skin was maintained at 32 °C during the whole experiment using a water bath. Before applying the formulations onto the skin, the skin was equilibrated at 32 °C for 15 min. In order to simulate an *in vivo* application, finite doses of formulations (10 μl/$cm^2$ or 10 mg/$cm^2$) were applied under non-occlusion. The experiment was carried out under light protection. The incubation time was 6 h. After 6 h a sample of 1 ml was withdrawn from the acceptor solution and analyzed for the mTHPC-content. Afterwards the formulations were removed from the skin by wiping the skin with cotton balls soaked with Ringers' solution. After cleaning, the skin was transferred onto a device for tape-stripping the SC.

#### 2.7.3. Stripping of the SC

The skin was stretched and mounted with pins on cork discs in the device for tape-stripping the SC. The skin was covered with a Teflon mask with a central hole of 15 mm in diameter. For removing the SC by tape-stripping, 20 strips, i.e. pieces of adhesive tapes (Cristall Klar Tesa, Beiersdorf AG, Hamburg, Germany) were used. Each tape-strip, previously weighed, was put onto the skin and a weight of 2 kg was put for 10 s onto the tape. Afterwards the tape-strip was rapidly removed with forceps, weighed and transferred into a glass vial of suitable size. Using Eq. (1) the weight of each SC layer was used to calculate the thickness of each SC layer (Michel et al., 1992):

$$T = \frac{d}{a \cdot p} \quad (1)$$

where $T$ is the thickness of removed SC (μm), $d$ is the difference in the weight of tape-strips before and after stripping (μg), $a$ is the stripping area (μm$^2$), and $p$ is the density of SC ($10^{-6}$ μg/μm$^3$). Using Eq. (1) the cumulative SC thickness was obtained.

Due to analytical reasons, the stripped tapes were collected in vials according to the following scheme: vial 1 = strip 1, vial 2 = strip 2–3, vial 3 = strips 4–5, vial 4 = strips 6–8, vial 5 = strips 9–12, vial 6 = 13–16, vial 7 = 17–20.

#### 2.7.4. Cryo-sectioning of the deeper skin layers (epidermis plus dermis)

After the tape-stripping the skin was frozen in liquid nitrogen and a skin disc of 10 mm was punched out from the stripped area. The skin disc was mounted with a drop of Tissue-tec O.C.T. (Sakura Finetechnical, Tokyo, Japan) onto a metal sample holder, which was transferred into the cryo-microtome (Reichert-Jung 2800 Frigocut, Leica Instruments GmbH, Nussloch, Germany). The skin was cut parallel to the surface into 25 μm thick layers. The skin sheets were put together in the following scheme: vial 1 = incomplete cuts, vial 2 = 10 × 25 μm, vial 3 = 10 × 25 μm, vial 4 = 20 × 25 μm and vial 5 = skin rest. The vials were weighed before collecting the skin cuts and afterwards. The mean value of the thickness per mg (μm/mg) was calculated using Eq. (2):

$$\text{mean thickness per mg}\,(\mu\text{m/mg}) = \frac{\sum \text{thickness of the cuts}}{\text{weight of the cuts}} \qquad (2)$$

Knowing the mean value of the thickness per mg (μm/mg) and the weight of the incomplete cuts and the skin rest, the thickness of the incomplete cuts and the skin rest was calculated.

### 2.8. HPLC assay of mTHPC

For the extraction of mTHPC from the tape strips and skin cuts methanol was used. The tape strips were extracted with 3 ml methanol, while the skin cuts were extracted with 1 ml methanol. The samples were shaken for 4 h at 60 °C under light protection. After that, 1–2 ml of the sample was centrifuged for 10 min at 13,000 rpm (Eppendorf miniSpin, Eppendorf AG, Hamburg, Germany). The supernatant was analyzed for the mTHPC-content by HPLC. The samples removed from the acceptor compartment after 6 h incubation time, were analyzed by HPLC directly. The HPLC system consisted of the solvent module “System Gold 126” (Beckman Coulter), autosampler “Triathlon” (Spark), fluorescence detector “RF-10A XL” (Shamdzu) with SS420x interface set for excitation wavelength at 410 nm and for emission wavelength at 654 nm, online degasser (ERC3415 alpha, ERC), column thermostat Jet-Stream Plus set at 30 °C (Thermotechnic Products), column LiChroCART250-4 with Purospher STAR RP-18 endcapped and guard column LiChroCART4-4 with Purospher STAR RP-18e endcapped (Merck). The mobile phase was composed of acetonitril:0.1% trifluoroacetic acid (TFA) in water (57.5:42.5, v/v) and the flow rate was set at 1 ml/min. The retention time for mTHPC was about 10 min and the injection volume was 50 μl. The measuring range was from 0.25 pg/μl to 100 pg/μl ($R$ = 0.9998) and the detection limit was 0.05 pg/μl. The software used was 32 Karat Software, Version 5.0, Build 1021 (Beckman Coulter). The tissue concentration of mTHPC was determined from a calibration curve constructed by plotting the peak height of mTHPC standard solutions. The calibration was linear within this range.

### 2.9. Statistical analysis

All measurements in the study were performed in triplicate and reported data present mean values ± S.D. Statistical analysis was carried out using the Student’s $t$-test. A $P < 0.05$ was considered statistically significant.

**Table 2**
Physico-chemical parameters of liposomes in dispersions and hydrogels and pH values of gels (mean ± S.D., $n$ = 3).

| Sample | Particle size (nm) | PDI | pH values |
|---|---|---|---|
| LDS-75 | 92.4 ± 0.3 | 0.172 ± 0.006 | 6.22 ± 0.02 |
| LG1S-75 | 87.7 ± 0.5 | 0.125 ± 0.014 | 6.77 ± 0.01 |
| LG2S-75 | 96.8 ± 0.3 | 0.122 ± 0.008 | 6.62 ± 0.01 |
| LG3S-75 | 97.1 ± 0.1 | 0.117 ± 0.005 | 6.94 ± 0.01 |
| LDS-100 | 91.3 ± 0.3 | 0.134 ± 0.008 | 6.35 ± 0.01 |
| LG1S-100 | 91.3 ± 0.4 | 0.128 ± 0.003 | 6.76 ± 0.01 |
| LG2S-100 | 91.1 ± 0.4 | 0.146 ± 0.006 | 6.63 ± 0.01 |
| LG3S-100 | 91.2 ± 0.8 | 0.172 ± 0.012 | 6.92 ± 0.01 |

## 3. Results and discussion

### 3.1. Characterization of liposomes

#### 3.1.1. Organoleptic characteristics, pH values and visualization of liposomes by cryo-electron microscopy

Liposome dispersions LDS-75 and LDS-100 were of a dark brown colour, transparent and without sediment. The pH determination (Table 2) revealed that both liposome dispersions expressed mild acid pH values, which did not differ significantly. Cryo-electron microscopy showed that the vesicles of both liposome dispersions were mostly bilamellar, and of a spherical and oval shape (Fig. 1a and b, arrows).

#### 3.1.2. Particle size and PDI of liposomes in liposome dispersions and liposomal gels

The results (Table 2) revealed a small particle size of liposomes in both liposome dispersions, which is desirable for a topical application (Verma et al., 2003b), and high homogeneity. In addition, the results indicated that the type of phospholipids used did not have a marked influence on the size and homogeneity of vesicles.

The incorporation of these liposomes into carbomer hydrogels of different polymer concentration did not lead to a remarkable change in the particle size (Table 2). The PDI values of liposomes in liposomal gels were lower or higher than the PDI values of liposomes in liposomal dispersions. However, they were small (PDI < 0.20), indicating homogeneous populations of vesicles. Hence, the liposomes in liposomal gels were of a small particle size and high homogeneity. Despite the fact that the polymer concentration in gels did not have a remarkable influence on the particle size of the incorporated vesicles, the particle size of liposomes in the gel LG1S-75 (0.5%, w/w, carbomer) was lower than in the gels LG2S-75 and LG3S-75 (0.75% and 1%, w/w, carbomer), and the liposome dispersion LDS-75 (used to prepare these gels), which indicated that the increased liposomes’ particle size in gels LG2S-75 and LG3S-75 was not a consequence of the higher polymer concentration. This observation was difficult to explain, since the particle size of liposomes in the gel LG1S-75 determined later every month during the 6 months’ stability study was in the expectable range from 95.9 ± 0.1 nm to 96.8 ± 0.8 nm (data not shown). This range of particle sizes was comparable to the liposome size in gels LG2S-75 and LG3S-75.

### 3.2. Characterization of liposomal hydrogels

#### 3.2.1. Organoleptic characteristics and pH values of liposomal hydrogels

All liposomal hydrogels were after their preparation of semisolid consistency as required for skin application, except the gel LG1S-100 which had a softer (lotion-like) consistency. All gels were

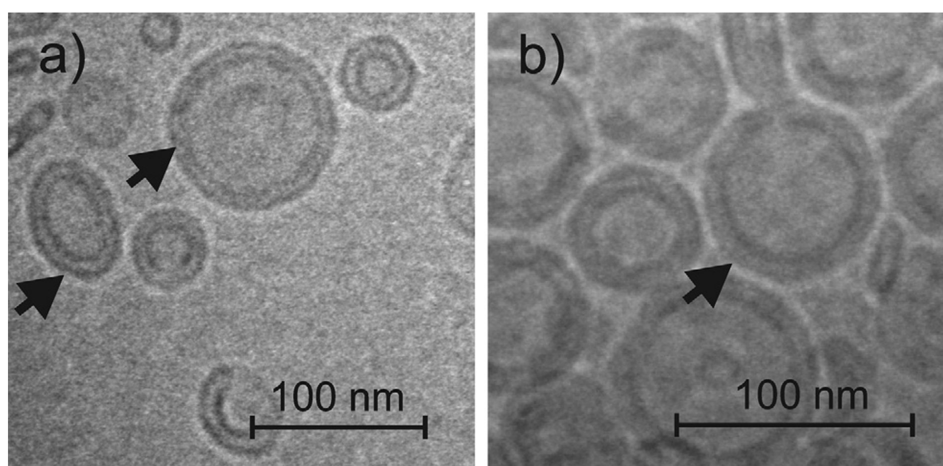


**Fig. 1.** Visualization of liposomes in mTHPC-loaded liposome dispersions by cryo-electron microscopy. (a) liposome dispersion LDS-75 and (b) liposome dispersion LDS-100. Arrows represent bilamellar vesicles.

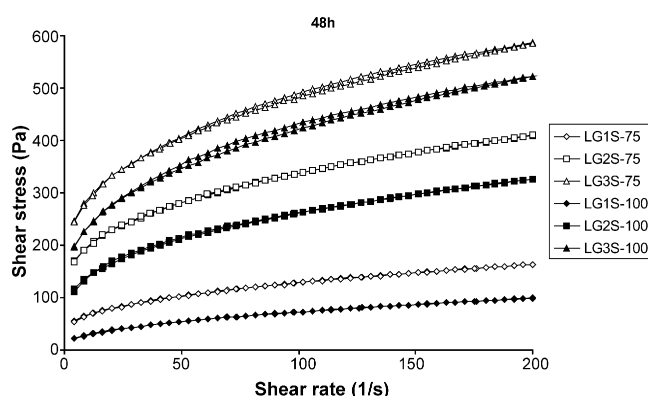


**Fig. 2.** Flow curves of liposomal gels obtained 48 h after preparation ($n = 3$).

transparent and of a dark brown colour. In addition, they were homogeneous and had a smooth and glossy texture. All hydrogels expressed a mild acid pH value acceptable for topical preparations (Table 2).

### 3.2.2. Rheological characterization of liposomal hydrogels

The flow curves of different liposomal gels (Fig. 2) revealed a non-Newtonian, plastic flow behaviour of samples, since they possessed yield values, indicating that the gel network exhibits resistance to an external force before it starts flowing (Briceno, 2000). The ascendant curves were analyzed using data analysis software, provided with the measurement equipment, and best fitting ($R > 0.999$) showed the Herschel-Bulkley model. The yield stress describes the flow behaviour at small shear rates, i.e. before and after the formulations' application. It is desirable for topical formulations to possess the yield stress, since these formulations show low resistance to flow when they are applied under high shear conditions, whereas at rest the flow is zero, which is appropriate for topical use (Bousmina, 1999). Therefore, formulations possessing the yield stress do not drip from fingers, spatulas and knives but hold their shape until sheared by spreading pressures which exceed their yield values, whereupon they flow and spread (Schott, 2000). The yield stress value can be used as an indicator of the formulations' stability since a good correlation was established between the yield value and elastic parameters (Tamburic and Craig, 1995a, 1996), which have been often used for the prediction of the long term stability of semisolid formulations (Ferry, 1980; Tamburic and Craig, 1996; Gasperlin et al., 1998).

The increase in the polymer concentration in liposomal gels led to an increase in the yield stress value and apparent maximal viscosity of gels (Table 3). Since the yield stress values increased from gel LG1S-75 to gel LG3S-75 and from gel LG1S-100 to gel LG3S-100, it is also expected that the stability of gels increased. Typical yield stress values of semisolid preparations are in the range 20–50 (80) Pa, dependent on the determination method, while values less than 20 Pa are considered to be characteristic for lotions. Therefore, sample LG1S-100, possessing the smallest yield stress value and considered to be a lotion, would start to flow when low external force is applied, and also after the application, i.e. at rest. This is not desirable for a topical application, since a certain contact time between the formulation and skin is required. In contrast, high yield stress values of samples LG2S-75, LG3S-75, LG2S-100 and LG3S-100 indicate higher resistance to an external force before the system starts flowing, and thus a greater degree of structuring (Ferry, 1980). The magnitude of yield stress relates to the strength

**Table 3**
Rheological parameters of liposomal hydrogels (mean ± S.D., $n = 3$).

| Sample | Yield stress[a] (Pa) | Maximal apparent viscosity[b] (Pa s) | Viscoelastic parameters[c] | | |
|---|---|---|---|---|---|
| | | | $G'$ (Pa) | $G''$ (Pa) | tan $\delta$ |
| LG1S-75 | 29.74 ± 0.57 | 13.37 ± 0.29 | 515.0 ± 24.0 | 159.0 ± 33.9 | 0.31 ± 0.08 |
| LG2S-75 | 106.35 ± 8.24 | 41.50 ± 0.66 | 1256.7 ± 81.4 | 235.7 ± 109.5 | 0.18 ± 0.07 |
| LG3S-75 | 141.85 ± 18.62 | 60.30 ± 1.87 | 1413.3 ± 75.1 | 314.0 ± 113.6 | 0.22 ± 0.07 |
| LG1S-100 | 9.40 ± 3.02 | 5.47 ± 0.39 | 198.5 ± 116.7 | 192.0 ± 87.7 | 1.01 ± 0.15 |
| LG2S-100 | 56.36 ± 4.65 | 28.53 ± 0.25 | 827.7 ± 78.8 | 330.7 ± 146.7 | 0.40 ± 0.18 |
| LG3S-100 | 80.40 ± 10.81 | 47.90 ± 0.75 | 1046.7 ± 40.4 | 276.0 ± 74.2 | 0.26 ± 0.07 |

[a] Yield stress was calculated using the Herschel-Bulkley regression mathematical model.
[b] Maximal apparent viscosity was determined by extrapolation from the ascendant flow curve at shear rate of 4.1 $s^{-1}$.
[c] Viscoelastic parameters were obtained in oscillatory measurements at the frequency of 1 Hz.

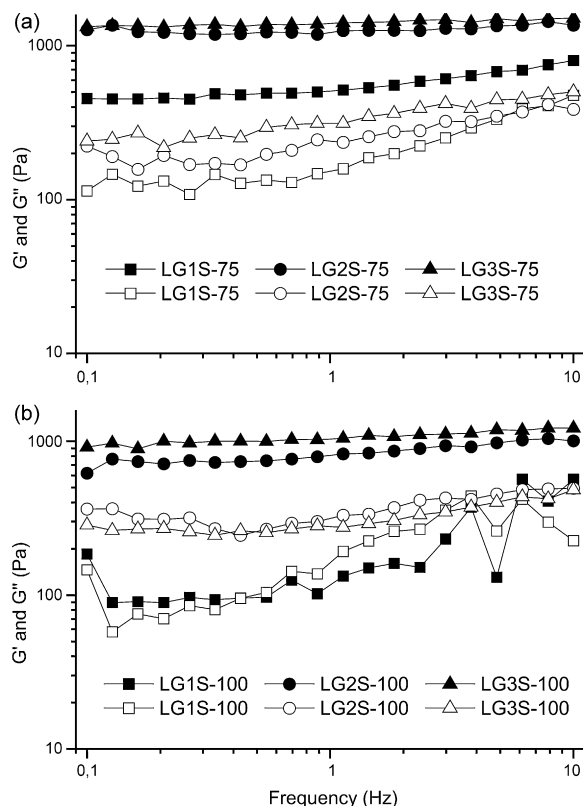


**Fig. 3.** Frequency sweep of liposomal gels. (a) Gels LG1S-75, LG2S-75 and LG3S-75, (b) gels LG1S-100, LG2S-100, LG3S-100. Full symbols represent the storage modulus $G'$, while empty symbols represent the loss modulus $G''$ ($n = 3$).

of the interparticle interaction of the three-dimensional network microstructure in creams (Adeyeye et al., 2002). High yield values may also represent the domination of the elastic behaviour, since a correlation was found between the yield stress and elastic parameters in some formulations (Tamburic and Craig, 1995a; Tamburic et al., 1998). This correlation was confirmed also in this study by oscillatory measurements (Fig. 3a and b, Table 3).

The viscoelasticity of gels was assessed by monitoring the storage (elastic) modulus ($G'$), viscous (loss) modulus ($G''$) and loss tangent (tan $\delta$). The storage modulus is a measure of the energy stored and recovered per cycle of deformation and reflects the solid-like component of viscoelastic behaviour, as opposed to the viscous modulus, which is a measure of the energy lost per cycle and reflects the liquid-like component (Ferry, 1980). The elastic modulus is known to be closely related to the connectivity of the polymeric network and quantitatively it is directly proportional to the number of entities which can support stress, i.e. elastically active network chains (Ross-Murphy and McEvoy, 1986). The loss tangent represents the ratio of the loss modulus and the storage modulus, and is another good indicator of overall viscoelasticity of the sample (Ferry, 1980; Ross-Murphy and McEvoy, 1986).

Liposomal gels LG1S-75, LG2S-75, LG3S-75, LG2S-100 and LG3S-100 revealed a domination of elastic over viscous behaviour, as the magnitude of their $G'$ was greater than that of the $G''$ (Fig. 3a). In addition, tan $\delta$, was less than 0.5 (Table 3). Values of tan $\delta$ smaller than 1 indicate the domination of elastic properties in the sample, i.e. high entanglements of particles (Gasperlin et al., 1998; Tamburic and Craig, 1995b). The values of both moduli increased with an increment in carbomer concentration (Table 3), indicating an increase in the elastic solid behaviour of gels due to the formation of more entanglements and more interactions in the polymer chains. However, in the case of the sample LG1S-100, values of $G'$ showed fluctuations which were dependent on the frequency, i.e. they were not higher than $G''$ at all frequencies. Therefore, this sample did not exhibit a predominant elastic behaviour, since at some frequencies the viscous behaviour was more dominant. This was also shown by the flow measurements, i.e. this sample possessed a very low yield stress value. At the frequency of 1 Hz, the tan $\delta$ was $1.01 \pm 0.15$, indicating equal contribution of $G'$ and $G''$ to the samples' viscoelastic behaviour.

In addition, liposomal gels containing the liposome dispersion LDS-75 possessed higher values of yield stress and viscosity, and also higher elasticity than gels containing the liposome dispersion LDS-100, indicating that the type of liposomes used had an influence on the flow and viscoelastic properties of gels. The liposome dispersions LDS-75 and LDS-100 differed in the purity of the phospholipids' mixture used for their preparation. The phospholipid mixture Lipoid S-75 contained, according to the manufacturers' certificate, a smaller amount of PC than the phospholipid mixture Lipoid S-100, but also contained glycolipids and sterol esters. The higher elasticity of gels LG1S-75, LG2S-75 and LG3S-75 could be, therefore, explained as a consequence of the presence of "impurities" in the liposome dispersion LDS-75, which led to a stronger gel network. However, this should be further investigated.

### *3.3. Penetration of mTHPC from liposomal gels into human skin*

In this study continuous flow measurements and also oscillatory measurements were performed, and put into correlation to the skin accumulation of mTHPC.

Continuous flow measurements were performed, since in most investigations the effect of viscosity on drug release was analyzed and inverse relations between viscosity and drug diffusion were found (Tas et al., 2004; Padamwar and Pokharkar, 2006). However, this method gives essential insights into the flow properties of samples (Tamburic and Craig, 1995a,b), and during this method samples are subjected to large deformations that may rupture or rearrange their structure, altering their rheological properties (Schott, 2000), which have influence on drug release and subsequent drug penetration (Bonacucina et al., 2004; A-sasutjarit et al., 2005). Therefore, in this study also dynamic measurements were conducted, because they involve small oscillatory deformations which are not changing the samples' structure (Schott, 2000). These tests allow direct assessment of viscoelastic properties (Tamburic and Craig, 1995a,b), which are more related to the intact structure of products than the viscosity (Schott, 2000), and should better correlate to release characteristics of a drug and its skin penetration (A-sasutjarit et al., 2005).

Due to the aforementioned advantages and disadvantages of the two methods, maximal apparent viscosity (as a parameter of flow measurements), and viscoelastic properties of gels (obtained by oscillatory measurements) were monitored and correlated with the skin penetration of mTHPC. Similar trends were observed in gels containing the liposome dispersion LDS-75 and gels containing the liposome dispersion LDS-100, i.e. the increment of the polymer concentration, which increased the viscosity and also viscoelastic parameters $G'$ and $G''$ (Table 3), decreased the drug accumulation in the SC (Fig. 4a, Table 4). Among all investigated gels, the gel LG1S-100, possessing the smallest apparent maximal viscosity and exhibiting a more viscous fluid behaviour than other gels, delivered the highest mTHPC-amount to the SC. The gel LG3S-75 exhibiting the highest maximal apparent viscosity and elasticity provided the smallest mTHPC-amount in the SC. This gel, and also other investigated gels (except LG1S-100), exhibited a domination of elastic over viscous behaviour, showing extensive entanglements between polymer chains, which could act as a fine mesh impeding the drug movement. Hence, higher viscosity of gels and the predominant elastic solid behaviour of gels decrease the drugs' pen-

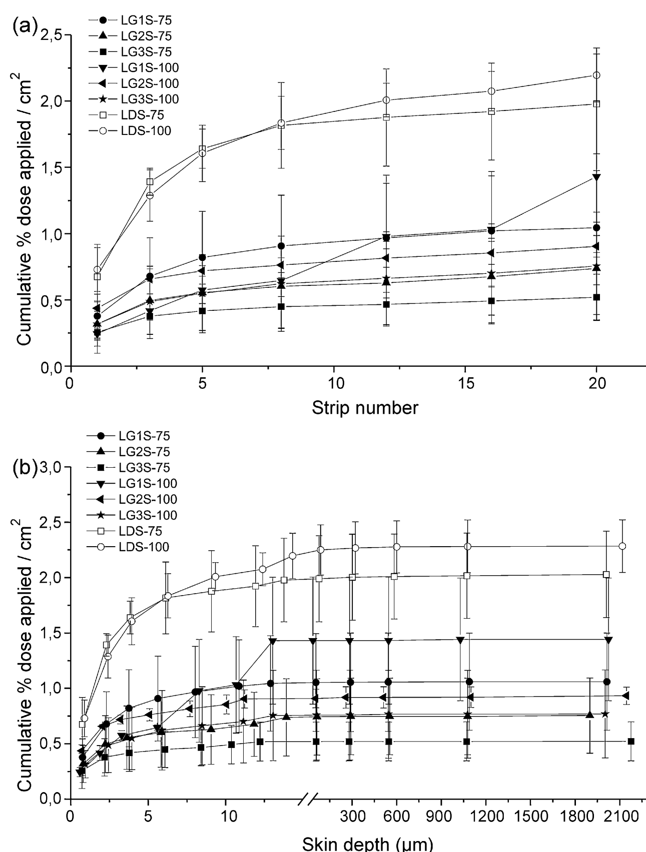


**Fig. 4.** Skin penetration of mTHPC after 6 h non-occlusive application of different liposomal gels onto human abdominal skin (expressed as cumulative % of dose applied/cm$^2$ ± S.D., $n$ = 3). (a) SC strip profile of mTHPC and (b) skin depth profile of mTHPC.

etration, which is in accordance with the findings of other authors (A-sasutjarit et al., 2005).

As to the deeper skin layers, the skin depth profile of mTHPC revealed that the increase in viscosity and elasticity of the formulations decreased the delivered mTHPC-amount (Fig. 4b, Table 4). However, there was not a corresponding relationship between these formulations' parameters and the accumulated mTHPC-amount. Among gels, the highest mTHPC-amount was delivered to the deeper skin layers by the gel LG2S-100, while the smallest mTHPC-amount was found upon the application of the gel LG3S-75 (Fig. 4a, Table 4).

On the basis of all results, the gel LG2S-100 containing 0.75%, w/w, carbomer providing sufficiently high mTHPC-amounts in the SC and the highest mTHPC-amounts (compared to other gels) in deeper skin layers (Fig. 4a, Table 4), possessed also desirable rheological characteristics, which would ensure the necessary contact time of the gel with the skin and also high stability. Therefore, this gel was considered to be the optimal formulation of mTHPC.

Regarding the therapeutic mTHPC-dose, which should be achieved in the skin tumor to induce its necrosis after the topical application of mTHPC, there are no available data. However, it was shown in mice bearing the subcutaneously (s.c.) located human colorectal tumor HT-29 that the required mTHPC-amount in the s.c. located tumor was between 0.105 and 0.050 ng mTHPC/mg wet tissue weight, depending on the light dose used in PDT, i.e. the amount decreased with increasing the light dose from 10 J/cm$^2$ to 50 J/cm$^2$ (unpublished data from biolitec AG, Germany). However, these data were obtained in mice bearing the s.c. located HT-29 tumor after an intravenous application of mTHPC. The HT-29 tumor is a very invasive tumor with an only intermediate sensitivity against PDT and therefore razes the needed doses of mTHPC (compared to skin cancers or psoriasis). Hence, it is not possible to give precise statements regarding mTHPC-doses required for the PDT of less invasive tumors, i.e. to exclude the possibility that the liposomal gel LG2S-100, which provided in the deeper skin layers an mTHPC-amount of 0.026 ng/mg tissue, could be used for the topical PDT of tumors in deeper skin layers. Therefore, our next step will be a PDT study in mice bearing a skin tumor.

Regarding liposome dispersions, they delivered higher mTHPC-amounts to SC and deeper skin layers than liposomal gels (Fig. 4a, Table 4). The liposome dispersion LD-S100 provided the highest mTHPC-amount in the SC and deeper skin layers. However, the application of liposome dispersions has its drawback, which derives from their liquid nature, i.e. low viscosity, which causes their leakage from the application site. Therefore, the liposome dispersions served only as controls in this study. Padamwar and Pokharkar (2006) found also a decrease in the skin deposition of drugs from gels as compared to the liposome dispersion, which might be due to increased viscosity of gels which retards the release from its structure. Mura et al. (2007) observed that the presence of the polymeric network in gels led to a slower drug release and lower permeation rate from liposomal gels compared to liposome dispersions.

Our finding that the increase in polymer concentration, i.e. increase in the maximal apparent viscosity in liposomal gels, leads to lower skin penetration of mTHPC (Tables 3 and 4) is in accordance with the results of Padamwar and Pokharkar (2006). Other authors (Tas et al., 2004; A-sasutjarit et al., 2005) also showed an inverse relationship between viscosity of gels and drug permeation. However, our study showed besides the good correlation between samples' viscosity and the skin penetration of the drug, also a correlation between samples' viscoelastic parameters and the skin penetration. Therefore, viscoelastic parameters which are related to the samples' intact structure should be correlated with the drugs' release and penetration (Padamwar and Pokharkar 2006). In contrast, flow measurements give essentially insights into flow properties of samples (Tamburic and Craig, 1995a,b) and are use-

**Table 4**
Amounts of mTHPC delivered from different mTHPC-formulations into different layers of human skin after 6 h non-occlusive application (mean ± S.D., $n$ = 3).

| Formulation | SC | | Deeper skin | | SC + deeper skin[a] | | SC | Deeper skin[a] |
|---|---|---|---|---|---|---|---|---|
| | [% dose/cm$^2$] | [ng/cm$^2$] | [% dose/cm$^2$] | [ng/cm$^2$] | [% dose/cm$^2$] | [ng/cm$^2$] | [% dose/cm$^2$] | [ng/cm$^2$] |
| LDS-75 | 1.98 ± 0.38 | 297 ± 57 | 0.055 ± 0.017 | 8.23 ± 2.56 | 2.03 ± 0.39 | 305 ± 58 | 217 ± 45 | 0.046 ± 0.009 |
| LG1S-75 | 1.04 ± 0.43 | 157 ± 60 | 0.016 ± 0.015 | 2.43 ± 2.26 | 1.06 ± 0.44 | 159 ± 61 | 120 ± 36 | 0.013 ± 0.013 |
| LG2S-75 | 0.74 ± 0.35 | 112 ± 51 | 0.017 ± 0.015 | 2.51 ± 2.23 | 0.75 ± 0.34 | 114 ± 50 | 81 ± 38 | 0.015 ± 0.014 |
| LG3S-75 | 0.52 ± 0.18 | 79 ± 29 | 0.002 ± 0.001 | 0.37 ± 0.20 | 0.52 ± 0.18 | 79 ± 29 | 64 ± 22 | 0.002 ± 0.001 |
| LDS-100 | 2.20 ± 0.20 | 329 ± 31 | 0.089 ± 0.051 | 13.31 ± 7.65 | 2.28 ± 0.24 | 343 ± 36 | 231 ± 16 | 0.074 ± 0.041 |
| LG1S-100 | 1.43 ± 0.57 | 195 ± 64 | 0.012 ± 0.019 | 1.82 ± 2.87 | 1.44 ± 0.55 | 197 ± 61 | 155 ± 66 | 0.011 ± 0.018 |
| LG2S-100 | 0.90 ± 0.08 | 130 ± 19 | 0.036 ± 0.019 | 5.07 ± 2.71 | 0.93 ± 0.08 | 135 ± 20 | 118 ± 27 | 0.026 ± 0.016 |
| LG3S-100 | 0.76 ± 0.41 | 116 ± 66 | 0.013 ± 0.012 | 1.99 ± 1.65 | 0.77 ± 0.40 | 118 ± 64 | 88 ± 45 | 0.011 ± 0.009 |

[a] Deeper skin means epidermis plus dermis.

ful for explaining the behaviour of the sample before and after its application and for predicting the contact time of the sample with the treated area.

## 4. Conclusion

The increase in the polymer concentration in liposomal gels led to an increase in viscosity and a predominant elastic solid behaviour of gels, which caused a decrease in the mTHPC-penetration into the skin. Among all formulations the liposomal gel containing the liposome dispersion LDS-100 and 0.75%, w/w, carbomer was considered to be the optimal formulation. This gel delivered a high amount of mTHPC into SC and also into the deeper skin layers, and it showed desirable rheological properties, which will ensure its sufficiently long contact time with the skin. Therefore, despite the fact that liposome dispersions deliver higher mTHPC-amounts into the skin, this study showed that it is also possible to deliver a sufficiently high mTHPC-amount into the skin by using a liposomal gel.

Hence, a mTHPC-loaded liposomal gel could be a promising tool for delivering the photosensitizer mTHPC into the skin, which would be advantageous for the PDT of cutaneous malignant (basal-cell carcinoma) or non-malignant diseases (psoriasis, acne, etc.).

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## References

Adeyeye, M.C., Jain, A.C., Ghorab, K.M., Reilly, W.J., 2002. Viscoelastic evaluation of topical creams containing microcrystalline cellulose/sodium carboxymethyl cellulose as stabilizer. AAPS Pharm. Sci. Technol., 3, article 8.

A-sasutjarit, R., Sirivat, A., Vayumhasuwan, P., 2005. Viscoelastic properties of Carbopol 940 gels and their relationships to piroxicam diffusion coefficients in gel bases. Pharm. Res. 22, 2134–2140.

Betz, G., Aeppli, A., Menshutina, N., Leuenberger, H., 2005. *In vivo* comparison of various liposome formulations for cosmetic application. Int. J. Pharm. 296, 44–54.

Biel, M.A., 2002. Photodynamic therapy in head and neck cancer. Curr. Oncol. Rep. 4, 87–96.

Bonacucina, G., Martelli, S., Palmieri, G.F., 2004. Rheological, mucoadhesive and release properties of Carbopol gels in hydrophilic cosolvents. Int. J. Pharm. 282, 115–130.

Boulmedarat, L., Grossiord, J.L., Fattal, E., Bochot, A., 2003. Influence of methyl-β-cyclodextrin and liposomes on the rheological properties of Carbomer 974P NF gels. Int. J. Pharm. 254, 59–64.

Bousmina, M., 1999. Rheology of polymer blends: linear model for viscoelastic emulsions. Rheol. Acta 38, 73–83.

Briceno, M.I., 2000. Rheology of Suspensions and Emulsions. In: Nielloud, F., Marti-Mestres, G. (Eds.), Pharmaceutical Emulsions and Suspensions. Marcel Dekker, Inc., New York, pp. 557–607.

Cevc, G., Blume, G., 1992. Lipid vesicles penetrate into intact skin owing to the transdermal osmotic gradients and hydration force. Biochim. Biophys. Acta 1104, 226–232.

Coderch, L., de Pera, M., Perez-Cullell, N., Estelrich, J., de la Maza, A., Parra, J.L., 1999. The effect of liposomes on skin barrier structure. Skin Pharmacol. Appl. Skin Physiol. 12, 235–246.

Dragicevic-Curic, N., Scheglmann, D., Albrecht, V., Fahr, A., 2008. Temoporfin-loaded invasomes: development, characterization and *in vitro* skin penetration studies. J. Control. Release 127, 59–69.

du Plessis, J., Weiner, N., Muller, D., 1994. The influence of *in vivo* treatment of skin with liposomes on the topical absorption of a hydrophilic and hydrophobic drug *in vitro*. Int. J. Pharm. 103, R1–R5.

El Maghraby, G.M.M., Williams, A.C., Barry, B.W., 2001. Skin delivery of 5-fluorouracil from ultradeformable and standard liposomes *in vitro*. J. Pharm. Pharmacol. 53, 1069–1077.

Ferry, J.D., 1980. Viscoelastic Properties of Polymers, 3rd edition. Willey, New York.

Fresno Contreras, M.J., Jiménez Soriano, M.M., Ramírez Diéguez, A., 2005. *In vitro* percutaneous absorption of all-trans retinoic acid applied in free form or encapsulated in stratum corneum lipid liposomes. Int. J. Pharm. 297, 134–145.

Gasperlin, M., Tusar, L., Tusar, M., Kristl, J., Smid-Korbar, J., 1998. Lipophilic semisolid emulsion systems: viscoelastic behaviour and prediction of physical stability by neural network modeling. Int. J. Pharm. 168, 243–254.

Glavas-Dodov, M., Goracinova, K., Mladenovska, K., Fredro-Kumbaradzi, E., 2002. Release profile of lidocaine HCl from topical liposomal gel formulation. Int. J. Pharm. 242, 381–384.

Harrison, S.M., Barry, B.W., Dugard, P.H., 1984. Effects of freezing on human skin permeability. J. Pharm. Pharmacol. 36, 261–262.

Hofland, H.E., van der Geest, R., Bodde, H.E., Junginger, H.E., Bouwstra, J.A., 1994. Estradiol permeation from nonionic surfactant vesicles through human stratum corneum *in vitro*. Pharm. Res. 11, 659–664.

Hopper, C., 2000. Photodynamic therapy: a clinical reality in the treatment of cancer. Lancet Oncol. 1, 212–219.

Kim, M.K., Chung, S.J., Lee, M.H., Shim, C.K., 1998. Delivery of hydrocortisone from liposomal suspensions to the hairless mouse skin following topical application under non-occlusive and occlusive conditions. J. Microencapsul. 15, 21–29.

Kirjavainen, M., Urti, A., Jääskeläinen, L., Suhonen, T.M., Paronen, P., Valjakka-Koskela, R., Kiesvaara, J., Mönkkönen, J., 1996. Interaction of liposomes with human skin *in vitro*-the influence of lipid composition and structure. Biochim. Biophys. Acta 1304, 179–189.

Kübler, A.C., Haase, T., Staff, C., 1999. Photodynamic therapy of primary non-melanomatous skin tumors of the head and neck. Laser Surg. Med. 25, 60–68.

Lasch, J., Laub, R., Wohlrab, W., 1991. How deep do intact liposomes penetrate into human skin? J. Control. Release 18, 55–58.

Michel, C., Purmann, T., Mentrup, E., Seiller, E., Kreuter, J., 1992. Effect of liposomes on percutaneous penetration of lipophilic materials. Int. J. Pharm. 84, 93–105.

Mura, P., Maestrelli, F., Gonzalez-Rodrıguez, M.L., Michelacci, I., Ghelardini, C., Rabasco, A.M., 2007. Development, characterization and *in vivo* evaluation of benzocaine-loaded liposomes. Eur. J. Pharm. Biopharm. 67, 86–95.

Padamwar, M.N., Pokharkar, V.B., 2006. Development of vitamin loaded topical liposomal formulation using factorial design approach: drug deposition and stability. Int. J. Pharm. 320, 37–44.

Puglia, C., Trombetta, D., Venuti, V., Saija, A., Bonina, F., 2004. Evaluation of *in vivo* topical anti-inflammatory activity of indomethacin from liposomal vesicles (LUV). J. Pharm. Pharmacol. 56, 1225–1232.

Ris, H.-B., Altermatt, J.H., Stewart, C.M., Schaffner, T., Wang, Q., Lim, C.K., Bonnett, R., Althaus, U., 1993. Photodynamic therapy with m-tetrahydroxyphenylchlorin *in vivo*: optimization of the therapeutic index. Int. J. Cancer 55, 245–249.

Ross-Murphy, S.B., McEvoy, H., 1986. Fundamentals of hydrogels and gelation. Br. Polym. J. 18, 2–7.

Schott, H., 2000. Rheology. Remington: The Science and Practice of Pharmacy, 20th edition. University of the Science, Philadelphia, USA, pp. 335–355.

Schreier, H., Bouwstra, J., 1994. Liposomes and niosomes as drug carriers: dermal and transdermal drug delivery. J. Control. Release 30, 1–15.

Seth, A.K., Misra, A., Umrigar, D., 2004. Topical liposomal gel of idoxuridine for the treatment of herpes simplex: pharmaceutical and clinical implications. Pharm. Dev. Technol. 9, 277–289.

Singh, R., Vyas, S.P., 1996. Topical liposomal system for localized and controlled drug delivery. J. Dermatol. Sci. 13, 107–111.

Tamburic, S., Craig, D.Q.M., 1995a. Rheological evaluation of polyacrylic acid hydrogels. Pharm. Sci. 1, 107–109.

Tamburic, S., Craig, D.Q.M., 1995b. An investigation into the rheological, dielectric and mucoadhesive properties of poly(acrylicacid) gel systems. J. Control. Rel. 32, 59–68.

Tamburic, S., Craig, D.Q.M., 1996. The effects of ageing on the rheological, dielectric and mucoadhesive properties of poly(acrylic acid) gel systems. Pharm. Res. 2, 279–283.

Tamburic, S., Vuleta, G., Simovic, S., Milic, J., 1998. Rheological evaluation of o/w cream bases containing polymeric emulsifier. In: Ziolkowsky, H. (Ed.), Emulsions. Verlag fur Chemische Industrie, GmbH, Augsburg, Germany, pp. 249–257.

Tas, C., Ozkan, Y., Savaser, A., Baykara, T., 2004. *In vitro* and ex vivo permeation studies of chlorpheniramine maleate gels prepared by carbomer derivatives. Drug. Dev. Ind. Pharm. 30, 637–647.

van Kuijk-Meuwissen, M.E.M.J., Junginger, H.E., Bouwstra, J.A., 1998. Interactions between liposomes and human skin *in vitro*, confocal laser scanning microscopy study. Biochim. Biophys. Acta 1371, 31–39.

Verma, D.D., Verma, S., Blume, G., Fahr, A., 2003a. Liposomes increase skin penetration of entrapped and non-entrapped hydrophilic substances into human skin: a skin penetration and confocal laser scanning microscopy study. Eur. J. Pharm. Biopharm. 55, 271–277.

Verma, D.D., Verma, S., Blume, G., Fahr, A., 2003b. Particle size of liposomes influences dermal delivery of substances into skin. Int. J. Pharm. 258, 141–151.

Wang, S., Kislalioglu, M.S., Breuer, M., 1999. The effect of rheological properties of experimental moisturizing creams/lotions on their efficacy and perceptual attributes. Int. J. Cosmet. Sci. 21, 167–188.

Zellmer, S., Pfeil, W., Lasch, J., 1995. Interaction of phosphatidylcholine liposomes with the human stratum corneum. Biochim. Biophys. Acta 1237, 176–182.