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Investigation of liposomes as carriers of sodium ascorbyl phosphate for cutaneous photoprotection

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Abstract

Long-term exposure of the skin to UV-A and UV-B radiation causes degenerative effect which can be decreased by scavenging reactive photochemical intermediates with antioxidants. In this study sodium ascorbyl phosphate (SAP), a very effective oxygen species scavenger, was encapsulated into liposomes in order to improve its penetration through the *stratum corneum* into the deeper layers of the skin. Two types of multilamellar vesicles were prepared, one from non-hydrogenated and the other from hydrogenated soybean lecithin, together with cholesterol, by the thin films method. They were characterized for size, polydispersity index, and ζ potential. In vitro diffusion of SAP and ex vivo penetration experiments were performed on pig ear epidermis membrane in a Franz diffusion cell. The size and ζ potential of liposomes containing SAP are significantly greater than those of empty liposomes. The upper limit of SAP entrapment efficiency was 8–10% in both types of liposomes. The stability of SAP in liposome formulations is much more influenced by storage temperature than by liposome composition. SAP penetrated through epidermis membrane significantly better from liposome dispersions than from water solution. The amount penetrating is much more influenced by the concentration of SAP in the formulation than by the lipid composition of liposomes. The SAP that penetrates through the epidermis reflects the active compound available to prevent or slow down the complex process of photodamage in the skin.

Keywords: Liposome; Photoprotection; Sodium ascorbyl phosphate; Skin; Drug delivery

1. Introduction

The uncovered parts of the skin are constantly exposed to solar UV irradiation. Long-term exposure to UV-A and UV-B radiation of the skin causes different degenerative effects as a consequence of complex biological processes (Wlaschek et al., 2001). Cellular changes and qualitative and quantitative alterations of dermal extracellular matrix proteins are involved. Absorption of photons by endogenous photosensitizer molecules follow subsequent reaction with oxygen resulting in the formation of different

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reactive oxygen species (ROS) including superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\bullet}$) and singlet oxygen ($^1O_2$). While low levels of ROS are produced continuously in vivo and are involved in physiological processes, there is accumulating evidence for their damaging effects at higher concentrations (Fuchs, 1998; Manček and Pečar, 2001). These involve severe oxidative stress in skin cells by interaction with intracellular chromophores and photosensitizers, resulting in transient and permanent genetic damage and activation of cytoplasmic signal transduction pathways that are related to growth, differentiation, replicate senescence and connective tissue degradation (Podda et al., 1998). Acute and chronic exposure to solar radiation has been linked to a number of types of skin damage such as sunburn, phototoxicity, photoallergy, aggravation of pre-existing skin diseases, distinct photodermatoses as well as to immunosuppression, photoaging, and photocarcinogenesis (Fuchs, 1998). In view of recent climate changes, the importance of this field is increasing daily.

General approaches to photoprotection aim at absorption or scattering of solar radiation (Seite et al., 2000; Nohynek and Schaefer, 2001; Pinnell et al., 2000). Another approach is to scavenge reactive photochemical intermediates (Jurkovič et al., 2003). The use of antioxidants to inhibit photooxidative toxicity has been suggested as an important strategy in preventing and treating photodamage. Nature achieves this by providing a numerous small lipophilic and hydrophilic skin antioxidants as well as enzymes and natural antioxidants. They have a low potential for side effects at physiological concentrations and appear to be particularly promising in photoprotection (Fuchs, 1998; Katiyar et al., 1999; Yamamoto, 2001). Of these, sodium ascorbyl phosphate (SAP) is one of the most effective free radical quenchers, and has the greatest potential for slowing down the detrimental effects resulting from photodamage (Jentzsch et al., 2001). SAP is a stable Vitamin C derivative, which is cleaved by enzymes in the skin to release ascorbic acid. It protects cells against free radicals, promotes collagen formation and acts on the melanine formation process (Austria et al., 1997). At physiological pH ascorbic acid is present mainly as the ascorbate anion. The latter penetrates poorly into the skin relative to the more lipophilic ascorbyl palmitate or other lipophilic antioxidants. For effective photoprotection it is desirable to reach an adequate antioxidant concentration of ascorbate in the target tissue, otherwise it could act as a pro-oxidant (Jurkovič et al., 2003). This goal can be achieved by delivering SAP into the skin by colloidal drug delivery systems (Barry, 2001); liposomes have the advantage of being able to convey substantial concentrations of small molecules across the skin barrier. They have been used successfully in enhancing the diffusion and clinical efficacy of a number of active ingredients (Barry, 2001; Betz et al., 2001; Fang et al., 2001; Cevc et al., 2002; Verma et al., 2003).

We report here results obtained using a formulation of SAP in liposomes, optimized to improve penetration of SAP through the *stratum corneum* into the deeper skin layers, and thus to provide internal UV protection of the skin.

2. Materials and methods

2.1. Materials

Sodium ascorbyl phosphate (SAP) was provided by BASF (Germany). Non-hydrogenated soybean lecithin (Phospholipon® 80) was obtained from Nattermann Phospholipid (Germany), and hydrogenated lecithin (Emulmetic® 320) from Lucas Mayer (Germany) and both were used without further purification. Cholesterol was supplied by Sigma Chemicals (USA), and hydrophilic cellulose membranes from Sartorius AG (Germany). All other chemicals and reagents purchased from commercial sources were of analytical grade. Water was freshly bidistilled.

2.2. Preparation of liposomes

Multilamellar vesicles (MLV) were prepared from non-hydrogenated (NS) or hydrogenated (HS) soybean lecithin in combination with 30% (w/w) of cholesterol by thin film method (Table 1). The organic solvent was evaporated and solvent traces were removed by maintaining the deposited lipid films under vacuum overnight. SAP was incorporated into liposomes during film hydration, at concentrations 1 and 2%. The hydration was performed above the phase transition temperature (T_m) of the phospholipids. T_m for NS is 23 °C, and for HS 80 °C. The dispersions of liposomes were downsized by sonication at predetermined values: son-

Table 1
The composition of liposome formulations

Formulation*	Components			
	Phospholipon 80	Emulmetic 320	Cholesterol	SAP (%)
NSL-0	+	–	+	0
NSL-1	+	–	+	1
NSL-2	+	–	+	2
HSL-0	–	+	+	0
HSL-1	–	+	+	1
HSL-2	–	+	+	2

* Phospholipid: cholesterol ratio was 70:30. The final lipid concentration in liposome formulations was 20 mg/ml.

ication time was 150 s and amplitude 35% (sonicator Cole-Parmer Ultrasonic Processor, USA).

2.3. Characterization of liposomes

Physical characteristics of liposomes in formulations (size, polydispersity index and zeta potential) were determined by photon correlation spectroscopy (PCS) and laser doppler anemometry (LDA). Samples were diluted with bidistilled water to a suitable concentration (fluctuation of light between 50 and 100 Kcts/s) and measured with a Zetaseizer 3000 (Malvern Instruments, UK). The measurements were repeated three to five times for each sample.

2.4. HPLC assay

The concentration of SAP was determined by HPLC utilizing a pump K-1001, UV detector K-2501 ($\lambda = 258$ nm, Knauer, Germany), autosampler "Midas", type 830 (Spark, Holland), degasser (Knauer, Germany) and a Nucleosil NH_2 column (100 μm, 250 mm × 4 mm). The mobile phase was a mixture of acetonitrile and 0.3 M phosphate buffer, pH 4 (40:60, v/v), and was delivered at a flow rate of 0.8 ml/min (Špiclin et al., 2001). The injection volume was 10 μl, and the retention time was found to be around 6 min. The column effluent was detected at 258 nm. The calibration curve for the quantification of SAP was linear over the range of concentration of 0.5–2.5 μg/ml with a correlation coefficient of R^2 of 0.999.

2.5. Entrapment efficiency

The entrapment efficiency (E.E.) in the liposomes was measured by determining the amount of SAP remaining after dialysis against distilled water. Samples were injected into a dialysis cell (Slide-A-Lyzer® Dialysis Casettes, Pierce USA) with a hydrophilic cellulose membrane (10,000 MWCO). After 4 h of dialysis, the dialyzing medium was changed and left overnight. After 24 h of dialysis, samples were withdrawn for HPLC analysis. Entrapment efficiency (%) was calculated from the ratio of the amount of SAP entrapped to the total amount of SAP used in the preparation of the liposome suspension (the reference solution was a sample that was not dialyzed). The experiment was repeated three times for each sample.

2.6. Stability studies

The physical stability of the liposomes and chemical stability of SAP were examined (Tables 2 and 3). All samples were stored in a well-closed 10 ml glass test tube at room temperature (22 ± 1 °C) in the dark, and in the refrigerator (5 ± 1 °C). Samples were taken immediately after preparation, then after 1, 2, 3, 7, 14, 28 and 56 days. The amount of undegraded SAP in samples was determined quantitatively by HPLC. Ten microlitres of 1% SAP liposome dispersion was diluted 1:100 (v/v) with mixture of tetrahydrofuran and

Table 2
Physical characteristics of liposomes: *z*-average mean size (*d*), polydispersity index (PI), zeta potential (ζ), and entrapment efficiency (E.E.) for SAP as mean ± S.D., $n = 3$–5

Formulation	*d* (nm)	PI	ζ potential (mV)	E.E. (%)
NSL-0	158.7 ± 2.7	0.43 ± 0.02	−46.4 ± 1.8	–
NSL-1	147.2 ± 4.3	0.36 ± 0.02	−67.5 ± 1.2	8.32 ± 0.03
NSL-2	166.4 ± 5.0	0.37 ± 0.03	−66.3 ± 0.6	9.94 ± 0.71
HSL-0	148.5 ± 1.7	0.29 ± 0.03	−48.0 ± 4.1	–
HSL-1	185.0 ± 4.0	0.39 ± 0.04	−66.1 ± 1.2	8.09 ± 0.45
HSL-2	205.9 ± 8.1	0.43 ± 0.03	−71.6 ± 4.7	9.23 ± 0.46

Table 3
The stability of SAP, expressed in percentages (w/w) of non-degraded SAP in NSL and HSL liposome formulations during stability studies period determined by HPLC in liposomes stored in the dark at 22 ± 1 °C, and in the refrigerator at 5 ± 1 °C

Formulation	Storage temperature (°C)	Storage time (Days)							
		0	1	2	3	7	14	28	56
NSL-1	5	100 ± 14.9	96.6 ± 17.9	96.2 ± 13.6	101.9 ± 15	99.2 ± 11.3	99.3 ± 2.2	97.5 ± 4.0	95.8 ± 6.3
NSL-1	22	100 ± 4.9	93.8 ± 5.5	93.6 ± 11.5	98.2 ± 5.5	96.9 ± 19.0	92.5 ± 4.9	94.9 ± 3.3	78.9 ± 3.4
HSL-1	5	100 ± 7.4	98.9 ± 8.4	99.4 ± 10.0	99.0 ± 3.4	100.1 ± 5.5	91.2 ± 9.5	98.4 ± 3.5	98.9 ± 8.6
HSL-1	22	100 ± 3.6	99.3 ± 5.5	96.2 ± 1.6	94.5 ± 7.3	99.2 ± 8.9	96.3 ± 5.8	94.3 ± 9.6	76.9 ± 12.4
HSL-2	5	100 ± 9.1	95.0 ± 9.8	100.9 ± 9.8	98.1 ± 4.8	98.9 ± 8.9	97.7 ± 5.7	98.2 ± 3.7	98.5 ± 4.2
HSL-2	22	100 ± 8.2	94.1 ± 13.0	97.1 ± 10.3	98.7 ± 8.3	101.0 ± 3.8	98.6 ± 8.0	96.8 ± 2.2	85.8 ± 4.8
NSL-2	5	100 ± 2.7	96.8 ± 4.2	96.6 ± 3.6	95.2 ± 8.7	98.3 ± 4.8	91.1 ± 3.1	93.1 ± 5.6	99.6 ± 13.6
NSL-2	22	100 ± 4.7	99.6 ± 4.3	95.4 ± 4.4	93.7 ± 5.7	95.7 ± 8.2	93.5 ± 6.7	90.5 ± 9.5	85.9 ± 6.7

0.3 M phosphate buffer, pH 4 (7:3), and then further diluted with 0.3 M phosphate buffer pH 4 to a final dilution 1:10,000 (v/v), followed by HPLC analysis as described in Section 2.4. Preparation of the 2% SAP dispersion differed only in the final dilution of 1:20,000 (v/v). Each experiment was repeated at least in triplicate.

2.7. In vitro diffusion study: membrane model

Drug diffusion profiles in vitro were determined at 37 ± 0.5 °C using a flow through diffusion cell (Sartorius–Membranfilter GmbH) with cellulose membrane, with phosphate buffer pH 7.4 as acceptor medium. Samples were taken at scheduled times intervals (30 or 60 min) and were analyzed by HPLC. Fifty microlitres of samples (solution and dispersion of liposomes) were diluted with 1:20 (v/v) with mixture tetrahydrofuran, 0.3 M phosphate buffer pH 4 (7:3), and then further diluted with 0.3 M phosphate buffer pH 4 to a final dilution 1:200 (v/v).

2.8. Ex vivo penetration study: Franz diffusion cell

Heat separated pig ear epidermal membrane was used for experiments of penetration using a cell, as proposed by Franz (1975). Before use, the skin is stored in refrigerator at −20 °C (most one month) was thawed for 1 h at room temperature, and skin disks with a diameter of 35 mm were punched. The disks were transferred to a water bath at 60 °C for 90 s. The epidermis was then carefully peeled off, using two forceps, and immersed in acceptor medium for 1 min, placed on filter paper to remove superfluous water, and sandwiched between two areas of ground glass with the *stratum corneum* side up. The available surface area for transport was 1.76 cm^2. The donor compartment contained 50 μl formulation (SAP solution or liposome dispersion) under occlusive conditions and the receiver compartment 7 ml of phosphate buffer pH 7.4. The diffusion cells were immersed in a water bath maintained at $T = 37 \pm 0.5$ °C, which resulted in a temperature on the skin surface of 33 °C. The receiver compartment was stirred by magnetic bar at 300 rpm. In control experiments, samples without SAP were used. At the beginning of each penetration experiment initial SAP concentrations were determined in the receiver and the donor compartment. The penetration experiments were carried out for 8 h. Two hundred microlitres samples were removed at appropriate intervals for HPLC analysis from the receiver compartment, which was immediately refilled with the same volume of fresh medium.

2.9. Statistical analysis

The results are given as arithmetic mean value ± standard deviation ($X \pm$ S.D.). Statistical comparisons were made using Student's *t*-test with $P < 0.05$ as the minimal level of significance.

3. Results and discussion

3.1. Characteristics of various liposome formulations

During the preparation, SAP was incorporated in liposomes at concentrations of 1% and 2%. The in-

fluence of SAP entrapment on the physical parameters of liposomes–particle size, polydispersity index and ζ potential—is shown in Table 2. Comparing the *z*-average mean size of liposomes with and without SAP shows significant differences (Student's *t*-test, $P < 0.05$). Variations are larger for HSL than for NSL liposomes. The liposomes containing SAP were larger in size than those without SAP presumably reflecting the different association properties of the phospholipids used. The influence of SAP incorporation on the value of polydispersity index (PI) is entirely different for NSL and HSL liposomes.

The ζ potential of the liposomes was in the range −46 to −72 mV, indicating adequate repulsive forces to prevent their fusion. Absolute values of ζ potential of the "empty" liposomes were smaller than loaded ones. The influence of SAP on the zeta potential values is significant and its absolute value being considerably greater than that of formulations without SAP. The zeta potential increase could be explained by the fact that the liposome surface is covered with ascorbyl phosphate, which bears a negative charge in the water environment. Increasing the concentration of ascorbyl phosphate to 2% did not cause any further significant difference (Student's *t*-test, $P > 0.05$) in the ζ potential. The negative value of ζ potential stays more or less unchanged on further increase of the SAP concentration in the liposome dispersion. These findings are in agreement with recently published results (Betz et al., 2001). They reported that the increased ζ potential in the presence of hydrophilic substances bearing negative charge is a consequence of two phenomena. Firstly, surface adsorption is a saturated process, which means that amount of SAP adhering to the liposome surface reaches a plateau. Secondly, ζ potential depends on the ionic strength of the solution, so that increasing the concentration of the ionic substances suppresses the ζ potential. During storage no appreciable variation of liposome size was detected by photon correlation spectroscopy (Fig. 1) and no drug precipitation or liposome aggregation was observed.

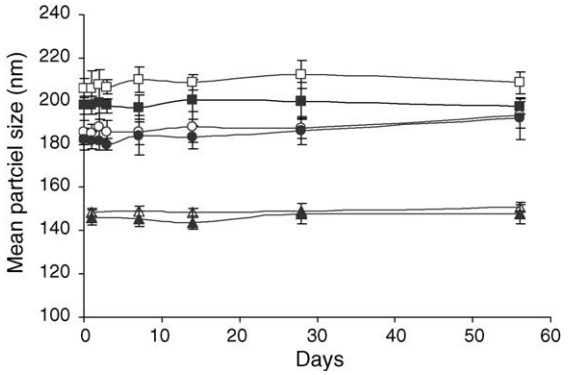

Fig. 1. Influence of the storage time on *z*-average mean size of HSL liposome suspensions: HSL-0 (△, ▲), HSL-1 (◯, ●) and HSL-2 (□, ■). Empty signes represent storage temperature at 5 ± 1 °C, full signes at 22 ± 1 °C.

3.2. Entrapment efficiency of SAP

Concentration has a significant influence on the entrapment efficiency for NSL and HSL liposomes (Student's *t*-test, $P < 0.05$) (Table 2). The entrapment efficiency is expressed as a percentage of encapsulated SAP in liposomes relative to the initial concentration. The influence of the type of phospholipid on entrapment efficiency is not significant ($P > 0.05$).

Encapsulation of approximately 8–10% of SAP is the upper limit in both non-hydrogenated and hydrogenated liposomes. This may be attributed to the preparation method used. The method of hydration constitutes an important obstacle to entrapment efficiency. The latter is relatively low for water-soluble agents (Zhang et al., 2001; Rengel et al., 2002). The reasons for low entrapment efficiency can also be found in the composition and charge of liposome lipids. Phospholipon® 80 (NS) or Emulmetic® 320 (HS) as soybean lipid extracts exhibit the negative charge of liposomes because of the negative phosphatidyl moieties that both contain (Table 2).

3.3. Stability

The stability of SAP was compared in the various liposome formulations (Table 3). It can be concluded that SAP in liposomes is more stable at lower temperature. During two months at 5 °C in the dark the percentage of undegraded SAP in the liposomes was almost unchanged at a mean value of 98.2 ± 8.2%; at 22 °C the equivalent mean value was 81.8 ± 6.9%.

No significant differences in stability profile were observed between SAP in NSL and HSL liposomes, independent of the amounts of SAP entrapped. These findings are in agreement with the observations of our

recent stability studies of ascorbyl palmitate, a derivative of ascorbic acid (Špiclin et al., 2001). SAP is one of the most stable derivatives of ascorbic acid in water because of its chemical structure. The phosphate group in the second position of the cyclic ring protects the enediol system of the molecule from oxidation (Austria et al., 1997; Špiclin et al., 2001).

3.4. In vitro diffusion study: membrane model

In order to obtain quantitative and qualitative information on SAP diffusion from the liposomes and to search for a correlation between their composition and the release mechanism, the complete diffusion profiles of SAP from liposomes and water solution were determined (Fig. 2). At both concentrations, more SAP diffuses from water solutions than from liposome suspensions. The release profiles from NSL and HSL liposomes did not differ significantly from water solution at the beginning (Student's *t*-test, $P > 0.05$), but at time points 300 and 480 min significantly lower percentages of released SAP were observed from liposomes ($P < 0.05$). Small differences between water solution and liposome suspensions in the first four hours of release studies are in accordance with the assumption that non-entrapped SAP was diffused first. After four hours, probably all free SAP is diffused from the medium and the difference is a consequence of the release of SAP from liposomes. Such results are the consequence of experimental conditions using hydrophilic cellulose membrane, through which dissolved SAP can diffuse easily. Liposome composition alone has no direct influence on the hydrophilic membrane. The very similar release profile from liposome suspensions is also due to the very similar amount of entrapped SAP (Table 2). Percentages of released SAP from 1 or 2% liposome suspensions did not differ significantly ($P > 0.05$), despite the absolute amount of released SAP from 2% formulations being two times larger.

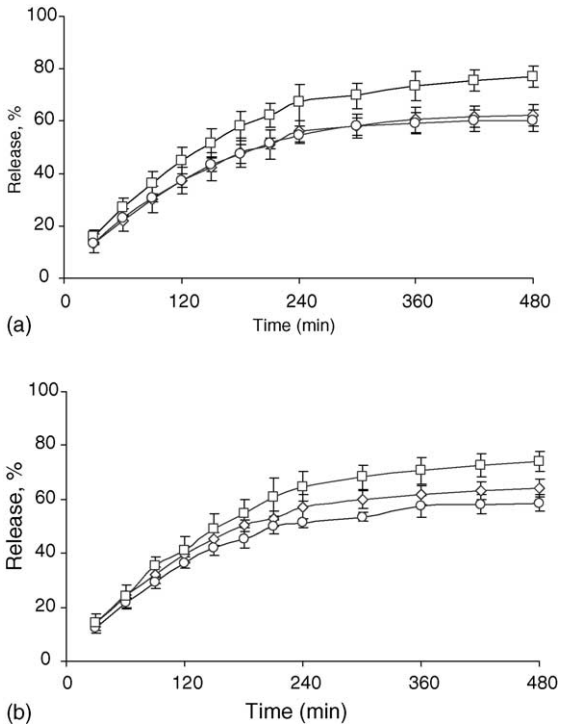

Fig. 2. Diffusion profiles of SAP through cellulose membrane from water solution (□) and from liposome dispersions prepared by NSL (◊) and HSL (○), containing (a) 1% of SAP and (b) 2% of SAP.

3.5. Ex vivo penetration study

The percutaneous penetration of SAP from water solution and liposome formulations was determined using a Franz cell. The aims of this study were to determine the penetration profile of SAP through epidermis when it is applied onto the skin as a water solution, to find whether entrapment of SAP in liposomes increases penetration through epidermis, and whether concentration and composition of liposome suspensions influence the penetration of SAP (Fig. 3). Ear skin from six different pigs was used. The greater variation in penetration comparing to the diffusion through cellulose membrane was expected to be due to inter-individual differences between skin donors.

3.5.1. Penetration of sodium ascorbyl phosphate from water solutions

The penetration profiles of SAP through epidermis are shown in Fig. 3. Concentration of SAP has a significant influence on the penetration from water solution (Student's *t*-test, $P < 0.05$). The mean cumulative penetration amount of SAP in eight hours for 1% solution is 18.6% and for 2% is 29.3% (Fig. 3). The higher penetration of SAP from 2% solution is probably due to higher concentration gradient.

These results indicate that SAP did, indeed, penetrate to a remarkable degree across the epidermis bar-

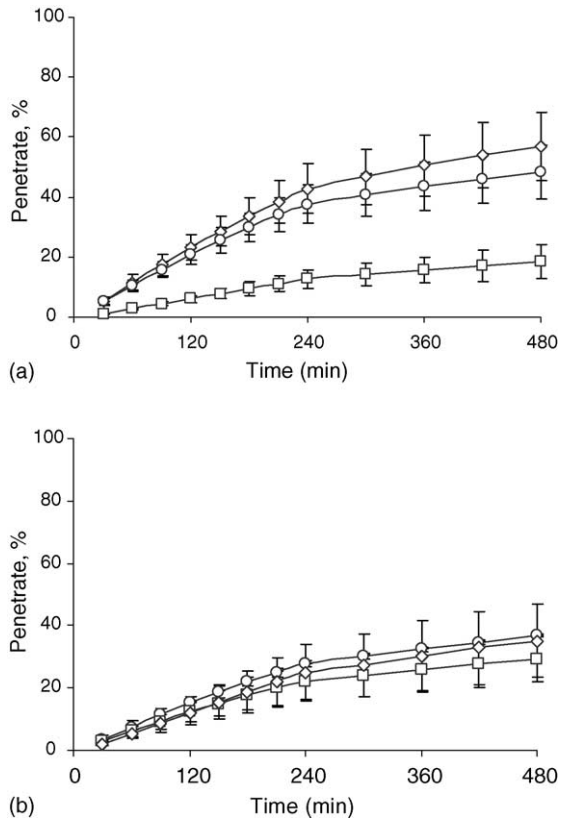

Fig. 3. Penetration profile of SAP through pig epidermis under ex vivo conditions from water solution (□) and from liposome dispersions prepared by NSL (◊) or HSL (○), containing (a) 1% of SAP and (b) 2% of SAP.

rier, although SAP is a water-soluble molecule. A significant lag-time was detected in penetration across the epidermis. This is in accordance with published studies, where it was demonstrated that sodium ascorbyl-2-phosphate and magnesium-L-ascorbyl-2-phosphate penetrate through the skin and are cleaved enzymatically to the biologically active antioxidant ascorbic acid (Jentzsch et al., 2001; Kobayashi et al., 1996). On the other hand, the ascorbic acid did not penatrate through dermatomized skin.

3.5.2. Penetration of sodium ascorbyl phosphate from liposome formulations

Our second aim was to determine if entrapment of SAP in liposomes improves penetration through epidermis comparing with water solution. Results are presented in Fig. 3. At 1% SAP the amount of penetrated compound was significantly higher from liposome formulations than from water solution (Fig. 3a). From NSL liposome formulations, almost three times as much SAP penetrated through epidermis (56.9 ± 11.4 versus 18.6 ± 5.7) than from water solution after 8 h of experiment; for HSL liposome formulations, the amount of SAP was 2.6 times higher (48.3 ± 8.8 versus 18.6 ± 5.7).

At 2% SAP the percent of penetrated SAP from NSL is 35.1 ± 11.9 and from HSL is 36.7 ± 0.5. This was a less evident increase in penetration over 2% of SAP water solution (Fig. 3b). According to prior results, this phenomenon has a logical explanation: at higher concentrations of drug, the rate of penetration decreases, because there are too many molecules trying to diffuse through the barrier, so they limit their own penetration (hindering effect) (Müller and Wissing, 2002). Nevertheless, the liposome formulation containing SAP in the medium and entrapped in the liposomes exhibits greater penetration through epidermal membrane than water solution. It is very important for effectiveness, as the level of free radical formed after radiation depends on the concentration of SAP available for scavenging and neutralising aggressive oxidizing agents and radicals (Jurkovič et al., 2004).

3.5.3. Influence of liposomes composition on SAP penetration

Penetration of SAP from NSL and HSL formulations was similar and not significantly different (Student's *t*-test, $P > 0.05$) (Fig. 3). Therefore, a composition-dependent penetration of drug as a consequence of phospholipid interactions with skin could not have been proven in this case. The discrepancy in the findings between these results and those of our previous experiments (Vrhovnik et al., 1998) and experiments carried out by Van den Bergh and co-workers (Van den Bergh et al., 1999) may be due to: (i) different experimental conditions or (ii) difference in physicochemical properties of the compounds used. Interactions of different liposome formulations with *stratum corneum* are reported to depend on the physical state of the phospholipid bilayers at the temperature of the experiment (Betz et al., 2001). Van den Bergh and co-workers (Van den Bergh et al., 1999) showed, by freeze-substitution electron microscopy that gel state liposomes aggregate

and adhere to the *stratum corneum* surface. However, liquid crystalline liposomes did not aggregate on the *stratum corneum* surface; these liposomes interact with intercellular lipids in *stratum corneum* layers and penetrate deeper into the skin. These differences are a result of the greater flexibility of the bilayer and freedom movement of the individual phospholipid molecules (Zellmer et al., 1995; Cevc et al., 2002). Thus, the liquid crystalline-state NSL liposomes should penetrate better through the skin than HSL gel-state liposomes. The reason why our results do not comply with the theory gel-state versus liquid crystalline state is that we incorporated 30% of cholesterol in the liposomes. Cholesterol exerts a considerable influence on the fluidity characteristics of liposomes and, hence, on their penetration behaviour (Coderch et al., 2000; Vrhovnik et al., 1998). Cholesterol increases the fluidity of the bilayer at temperatures below the phase transition temperature (T_m), and decreases the fluidity at temperatures above T_m. Since the epidermal membrane temperature is above the T_m of NSL, cholesterol should decrease the bilayer fluidity. On the other hand, the epidermal membrane temperature is below the T_m of HSL, so cholesterol increases its fluidity. So, the absence of differences in penetration of SAP from NSL and HSL liposomes suspensions can be attributed to the effects of cholesterol on the fluidity of the liposome bilayer.

4. Conclusion

From the present study it can be concluded that liposome carriers enable greater SAP penetration through the epidermal membrane than when applied SAP in water solution to the skin surface. The difference in liposome composition did not have any significant effect on the penetration of SAP when present entrapped, or non-entrapped and applied topically in liposome suspension. At higher concentrations of SAP, the absolute amount of SAP penetrated was larger. The penetration profile is much more influenced by concentration of drug than the liposome composition. The epidermal membrane experiments support the claim that the SAP that penetrated through the epidermis reflects the active compound, which could slow down the complex process of phototoxicity to the skin.

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