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Liposomal Encapsulation of Photoprotector Molecules

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Research Article

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ABSTRACT

Context: As the amount of sunscreen agent inside the stratum corneum has a direct relationship with its sun protection value, the formulation of drug carrier systems for the encapsulation and delivery of photoprotective molecules have an importance. On this basis the unique property of the liposomes can be used for the encapsulation of ultraviolet filter molecules and the development of liposomal sunscreen agents.

Methods and Material: In the present study we investigated four different lipid compositions from L-α-phosphatidylcholine (DPPC), Cholesterol (CHOL), 1,2-Di-O-Octadecenyl-3-Trimethylammoniumpropane chloride salt (DOTMA) and 1,2-Dioleoyl-sn-glycero-3-phosphocholine (DOPC) for the encapsulation of three ultraviolet filters – octocrylene, avobenzone and phenyl salicylate.

Settings and Design: We evaluated, whether the charge and fluidity of the bilayer, the cholesterol content of the liposomes and the lamellarity of the vesicles have an impact on the encapsulation efficiency of the selected photoprotector molecules.

Results: As ultraviolet filters possess lipophilic character, the liposomal encapsulations resulted in high encapsulation efficiency values – not significantly depending on the composition and lamellarity of lipid vesicles. Our promising results can be used for design of sunscreens possessing advantageous pharmacokinetic and cosmetic properties.

INTRODUCTION

Sunscreens are designed to protect the skin from the harmful effects of solar radiation, particularly the ultraviolet (UV) band roughly divided into two segments according to wavelength, the short-wavelength UVB (290-320 nm) and the long-wavelength UVA (320-400 nm). The effect of topical sunscreens is most commonly based on substances that absorb the UV photons thus preventing their incidence on the cells of the skin acceptors [1].

One of the oldest chemical absorbers salicylates, such as phenyl salicylate (PS) is a weak UVB absorber [2]. Nowadays, one of the most commonly used photoprotector, avobenzone (AVO) provides superior protection through a large portion of the UVA range [2]. Contrary to AVO, the absorption profile of octocrylene (OCTO) spans from 290 to 360 nm, having an excellent safety profile with low irritation, phototoxicity and photoallergic potential [2,3] **(Figure 1)**.

For an (UV) filter containing sunscreen to be effective, the UV absorber molecules must remain in the outermost region of the skin, as the amount of sunscreen agent inside the stratum corneum has a direct relationship with its sun protection value [4-6]. Thus, an ideal sunscreen product should exhibit high skin accumulation of the photoprotector molecules with minimal permeation to the circulation [4]. Topical sunscreens are presented as ointments, lotions, creams and sprays. Beyond their photoprotective activity –

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expressed as sun protection factor (SPF) - cosmetic properties, like color, stiffness, texture, tackiness etc., being important from the aspect of use should also take into consideration.

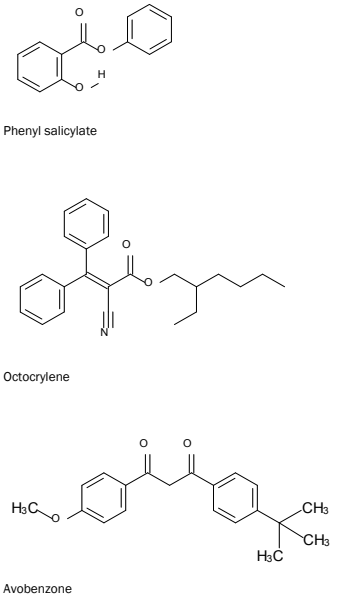

Figure 1. Chemical structures of phenyl salicylate, octocrylene and avobenzone.

By choosing innovative delivery system for the UV-filter and optimizing it, not only sunscreen products can be significantly improved in terms of pharmacological efficacy and cosmetic appearance but the potential toxicological risk associated with the systemic effects of UV filters may be reduced [6-8]. For many drugs it has been shown that liposomal encapsulation of drugs produces several-fold higher drug concentrations in the epidermis and dermis, with lower systemic concentrations compared to those in the conventional dosage forms (e.g. gel, lotion, ointment) [1,4]. On this basis the unique property of the liposomes can be used for the encapsulation of photoprotector molecules and the development of liposomal sunscreen agents being superior to conventional products.

MATERIALS AND METHODS

The lipids used in these experiments were 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-dioleoyl-Sn-glycero-3-phosphocholine (DOPC) and 1,2-di-O-octadecenyl-3-trimethylammonium propane (chloride salt) (DOTMA) from Avanti Polar Lipids Inc. (Alabaster, Al, USA). Cholesterol was purchased from Sigma Aldrich (St. Louis, MO, USA). Absolute ethanol and chloroform (Sigma Aldrich, St. Louis, MO, USA) were used to dissolve the lipids. Sodium phosphate, monobasic and dibasic (J. T. Baker, Phillipsburg, NJ, USA) were used to prepare the buffer solutions. Octocrylene (2-ethylhexyl 2-cyano-3,3-diphenyl-2-propenoate) and avobenzone (4-tert-butyl-4'-methoxydibenzoylmethane or parsol 1789) were ordered from Sigma Aldrich, St. Louis, MO, USA, phenyl salicylate was purchased from Hungaropharma Ltd., Budapest, Hungary.

Preparation of liposomes

The lipid films were prepared by thin-film hydration technique. The appropriate amounts of lipids (as given in **Table 1**) were dissolved in the mixture of chloroform and ethanol (2:1 v/v). The solvents were evaporated using Buchi rotavapor R-210 ordered from Sigma Aldrich, St. Louis, MO, USA for 60 minutes, under inert (nitrogen) atmosphere for the first 20 minutes without vacuum at 45°C. The lipid films were stored in a desiccator overnight at room temperature. In order to produce multilamellar vesicles (MLV), dry lipid films were hydrated with the UV filter-containing phosphate buffers (pH=5.3) at 52°C, resulting in lipid to UV filter ratios of ~10 to 1. After the addition of hydrating solution the round bottom flask was vortexed to ensure the homogeneity of the mixture. Small unilamellar vesicles (SUV) were prepared from MLVs by extrusion (Avanti mini extruder, Avanti Polar Lipids Inc., Alabaster, Alabama, USA) through polycarbonate filters (Avanti Polar Lipids Inc., Alabaster, Alabama, USA) with pore sizes of 400 and 200 nm.

Measurement of encapsulation efficiency

Freshly prepared UV-filter containing MLVs and SUVs were centrifuged with the Galaxy 16DH Eppendorf centrifuge (2 × 10 min, 13,000 xg) through Nanosep 10K Omega Centrifugal Filter Devices (PALL Life Science Inc.) with a cut-off value of 10 kDa to separate the liposomes from the outer solution. The encapsulation efficiencies for octocrylene, avobenzone and phenyl salicylate were determined by spectrophotometry (Genesys 10 UV spectrophotometer) at wavelengths of 300, 357 and 309 nm, respectively. The absorbance values of the photoprotector containing hydrating solutions (Absi) and the absorbance of the filtered outer phase of liposomal samples (Absf) were determined. The encapsulation efficiency was determined using the following equation.

$EE=(Ab_{si}-Ab_{sf})/Ab_{si} \times 100$

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Table 1. Determination of encapsulation efficiency (%) for avobenzone and octocrylene in SUVs and MLVs with various compositions; for hydration phosphate buffer (pH 5.3) was used (n=3). In each case the lipid concentration was 2.0 mg/ml, the avobenzone and octocrylene concentrations were 0.1 mg/ml, the phenyl salicylate concentration was 0.06 mg/ml.

Liposomal composition		EE (%) (mean ± S.D.)	
		MLV	SUV
Avobenzone	DPPC	99.60 ± 1.01	99.20 ± 0.89
	DPPC/CHOL (70/30 w/w)	100.16 ± 1.05	100.08 ± 0.09
	DPPC/DOTMA (97/3 w/w)	99.76 ± 0.44	99.76 ± 0.58
	DPPC/DOPC (70/30 w/w)	99.84 ± 0.13	99.52 ± 0.22
Octocrylene	DPPC	98.97 ± 1.10	98.81 ± 0.34
	DPPC/CHOL (70/30 w/w)	99.52 ± 0.07	99.52 ± 0.56
	DPPC/DOTMA (97/3 w/w)	97.70 ± 1.12	98.18 ± 0.77
	DPPC/DOPC (70/30 w/w)	99.13 ± 0.31	99.45 ± 0.20
Phenyl salicylate	DPPC	94.16 ± 2.38	93.28 ± 2.11

Zeta potential measurements

Zeta potential measurements were carried out at 25°C by Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK) on SUVs. 500 µl of SUV samples with 2 mg/ml lipid concentration was diluted with an appropriate volume of the phosphate buffer and adjusted 1 ml final volume.

RESULTS AND DISCUSSION

The liposomal encapsulation of avobenzone, octocrylene and phenyl salicylate resulted in high encapsulation efficiency values **(Table 1)**. The encapsulation efficiency values were near to 100%. For AVO and OCTO in average they were higher than 97.7%, while in case of PS slightly lower values were determined, being between 93-94%. According to statistical analysis the encapsulation efficiency values for AVO and OCTO did not differ significantly ($p>0.05$) from each other. However, the encapsulation efficiency values for PS were slightly, but significantly lower.

In general, partition coefficient, logPo/w, is used to characterize distribution of drugs between lipophilic and aqueous phases. There is, however, also a general agreement that a simple use of the logP values for liposomal suspension can result in deviations from the expected partition due to molecular interaction that can impact the phenomenon [9]. Hydrophobic molecules – as the photoprotector molecules, used in this study - in the right liposomal formulations could potentially have encapsulation efficiency values nearing 100%, and their location is supposed to be among the lipophilic fatty acid chains. Among the three photoprotector molecules OCTO possesses the highest logP value, while it is the lowest for PS [10], being in agreement with the slightly lower encapsulation efficiency data gained for PS **(Table 2)**.

Table 2. logP values of the photoprotector molecules [10].

Photoprotector	logP
Avobenzone	3.728
Octocrylene	7.039
Phenyl salicylate	2.125

In case of DOTAP-containing liposomes the positive surface charge of the vesicles does not have a significant impact on the encapsulation of the UV-filters, allowing to suppose that surface processes and molecular interactions between the lipid head groups do not play a significant role in the encapsulation process of UV filter molecules.

Regarding the high encapsulation efficiency values the literature data coincide with our observations. Golmohammadzadeh and his co-workers prepared octyl methoxycinnamate (OMC) containing liposomes from soy lecithin, cholesterol, vitamin E and propylparaben [4]. The encapsulation efficiencies for MLV and SUV liposomes containing OMC were 89.66 ± 2.08% and 89.7 ± 0.7%, respectively. The location of OMC is supposed to be in the hydrophobic interior of the liposomal bilayer due to OMC's hydrophobic characteristics. According to their results MLV formulations delivered significantly higher amounts of OMC to the stratum corneum and reduced the penetration of UV-filter to the deeper layers at the same time than conventional lotion and SUVs [4,11]. Finally, the SPF of the liposomes containing OMC was higher than that of the control lotions at a similar concentration of OMC [4].

In another study the encapsulation of AVO in liposomes using isolecitine was found to be highly efficient, roughly 90 % [12].

Beyond the high encapsulation efficiencies of UV filter the liposomal form offers other advantages, too. The physiologic pH of the liposomal systems -5.91 ± 0.35 for our samples - does not harm the skin. The viscosity of vesicular systems is higher than that of solutions resulting in better applicability. Furthermore, liposomes make possible to design products without oily texture, which can only hardly be reached with traditional forms of sunscreens.

Regarding the stability of liposomal samples the zeta potential measurements are promising. According to our preliminary measurements the zeta potential of SUVs prepared at pH=5.6 from soy lecithin DPPC is -45.52 ± 1.1 mV. The presence of photoprotectors, as being localized between the fatty acid chains, does not seem to significantly influence the zeta potential values determined. Since the absolute values of the measured zeta potentials are above the theoretically appointed 30 mV limit required for stability, we can conclude that the use of such liposomes is acceptable for the preparation of stable liposomal formulation [13]. However, the detailed stability characterization of liposomal photoprotectors is beyond the scope of the present work. As UV filters possess lipophilic character, the liposomal encapsulations resulted in high encapsulation efficiency values-not significantly depending on the composition and lamellarity of lipid vesicles. It can be supposed that UV filter molecules are localized between the fatty acid chains in the liposomal bilayer.

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