J. Appl. Cosmetol. **24,** 139-147 (October/December 2006)

NEW APPROACHES FOR UV-INDUCED PHOTODAMAGE PROTECTION

Elka Touitou and Biana Godin
Department of Pharmaceutics, School of Pharmacy, Faculty of Medicine, The Hebrew University of Jerusalem, Israel

Received: *August 2006*

Key words: *Sunscreens; Jojoba derivatives; Ethosomes; Alpha-tocopherol; Skin photodamage;*

Summary

This paper describes a dual approach for efficient protection from UV-induced skin damage: (1) use of a new generation of chemical sunscreens acting on the skin surface and lacking transdermal absorption followed by (2) use of ethosomes for enhanced dermal and intracellular delivery of free radical scavengers.

The basic concept behind the design of Skin Non-Permeating SUNscreens (NPSUN) was to immobilize UV absorbing moieties in the structure of a jojoba oil backbone. NPSUNs containing two or four methoxycinnamic UV absorbing groups (MC-NPSUNs) were synthesized and characterized. No permeation of MC-NPSUNs across the skin was observed in 24 hour *in vitro* permeation experiments either following application of neat compounds or after application of MC-NPSUNs formulated in o/w cream, in o/w cream or in jojoba oil. An additional valuable feature of NPSUNs is their high skin substantivity which could minimize the need for repetitive applications.

Ethosomes, specially designed carriers for enhanced dermal and intracellular delivery, were used to improve uptake of alpha-tocopherol into the skin cells for prevention of malignant processes induced by solar radiation. The results show that alpha-tocopherol was efficiently delivered into the skin and across the cellular membranes from ethosomal systems.

Impediment of sunscreen systemic absorption diminishes the potential of sunscreens to cause systemic adverse reactions, while the efficient delivery of alpha-tocopherol to the site of its action within the skin cells increases the agent's efficiency in inhibiting sun related carcinogenic processes.

Riassunto

Questo studio riporta il duplice approccio possibile per ottenere una efficiente protezione nei confronti dei danni cutanei provocati dagli UV (1). L'utilizzazione di una nuova generazione di filtri in grado di migliorare il rilascio di attivi capaci di neutralizzare i radicali liberi.

Il concetto base per impedire la penetrazione dei filtri solari (NPSUN) è quello di adsorbirli nella struttura base dell'olio di jojoba (NPSUN). Così sono stati sintetizzati e caratterizzati NPSUN con-

tenenti 2 o 4 gruppi metossicinnamici (filtri UV). E' stato così visto che questo particolare veicolo (NPSUN) dopo 24 ore non penetra attraverso la cute se inserito sia in emulsioni o/a che a/o o in olio di jojoba. Inoltre ne è stato anche valutato il grado di sostantività (affinità per la pelle) in modo da evitare la ripetuta applicazione del prodotto. Al contrario, per facilitare la penetrazione dell'a-tocoferolo (vit. E) attraverso la pelle in modo da prevenire i danni da UV, sono stati utilizzati gli etosomi. Dai risultati ottenuti si desumono chiaramente come gli etosomi siano in grado di penetrare facilmente attraverso gli strati cutanei e le membrane cellulari.
L'impedita penetrazione dei filtri solari ne impedisce gli effetti negativi sistemici, mentre la penetrazione degli etosomi con la vit. E inibisce l'instaurarsi dei fenomeni carcinogenetici legati all'azione degli UV:

INTRODUCTION

It is well accepted that there is a high need to protect our skin from UV induced photodamage and photocarcinogenesis [1, 2]. This paper presents a dual approach for efficient skin photoprotection by proposing sunscreens that do not permeate the skin on the one hand, and a carrier for enhanced skin penetration of free- radical-scavengers on the other hand (Figure 1).

New Skin Non-Permeating Sunscreens (NPSUN)

Chemical sunscreens are prophylactic agents that absorb UV radiation on the surface of the skin. Until now, sunscreen manufacturers have convinced regulators, formulators, and consumers that protection from UV-induced skin photodamages outweighs any supposed risk from the sunscreen agents. However, for many years researchers have been aware of the possible toxicity caused by percutaneous absorption of chemical sunscreens topically applied to the skin. More recent reports support this concern [3, 4]. Widely used sunscreens, such as octylmethoxycinnamate (OMC) and benzophenone-3, were reported to have an estrogenic effect in laboratory tests, causing cancer cells to grow more rapidly and triggering developmental abnormalities in rats [5, 6].

The currently used chemical sunscreens are generally small organic lipophilic molecules, which due to their physico-chemical characteristics possess a good potential to penetrate into the deep strata of the skin and to be systemically absorbed. Unfortunately, the *in vitro* skin permeation tests often use inadequate experimental conditions, e.g. receiver medium, that do not allow clearance of the lipophilic sunscreen from the skin layers into the receiver diffusion cell.

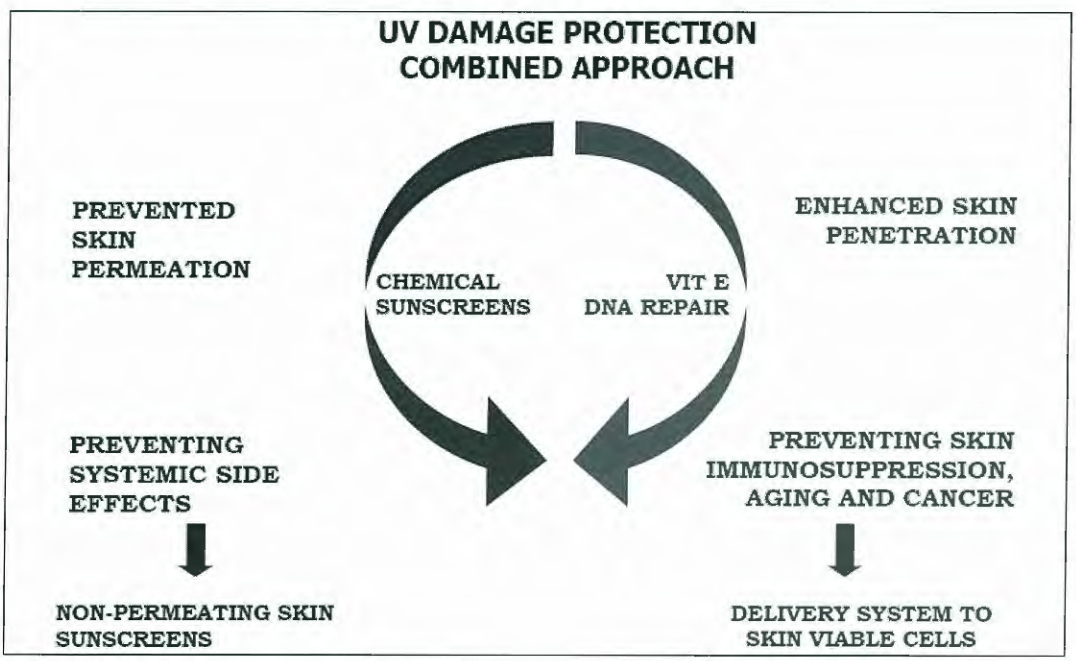

Fig. 1 Combined approach for efficient protection from UV induced skin damage.

This can explain why many reports in the literature support the claim that sunscreen molecules do not penetrate the skin. On the other hand, when an appropriate receiver vehicle was used 9% of the applied dose (10mg) of the widely used sunscreen OMC permeated across the skin with a flux 27μg/cm^{2}h (Figure 2).

It is important to keep in mind that sunscreens are applied to a large skin area (≥1 m^2) and for a long period of time, producing a constant and significant absorption of the sunscreen into the systemic circulation. Thus there is a critical need for the development of new products based on skin non-penetrating photoprotectors.

With the aim of introducing new sunscreen molecules that have an advantage over currently marketed ones, we designed skin Non-Permeating SUNscreens (NPSUN). The basic concept behind these molecules was to immobilize UV absorbing moieties in the structure of a jojoba oil backbone [7]. For this purpose NPSUN jojoba oil-UV sunscreens were designed and investigated. Jojoba oil is an ester of fatty acids (C18-22) with fatty alcohols (C18-22), which is widely used as an ingredient in cosmetic and pharmaceutical products [8]. This oil was chosen to serve as a backbone for the new derivatives for two main reasons: it has a sufficiently high molecular weight (~600-700) and a high lipophilicity. These physico-chemical characteristics cause jojoba oil and its derivatives to accumulate within the upper skin layer, the stratum corneum, the site of action of the sunscreen molecule. Each jojoba oil molecule could be linked by ester bonds to two-four UV absorbing units and UVA, UVB or UVA-UVB NPSUNs could be designed (Figure 3).

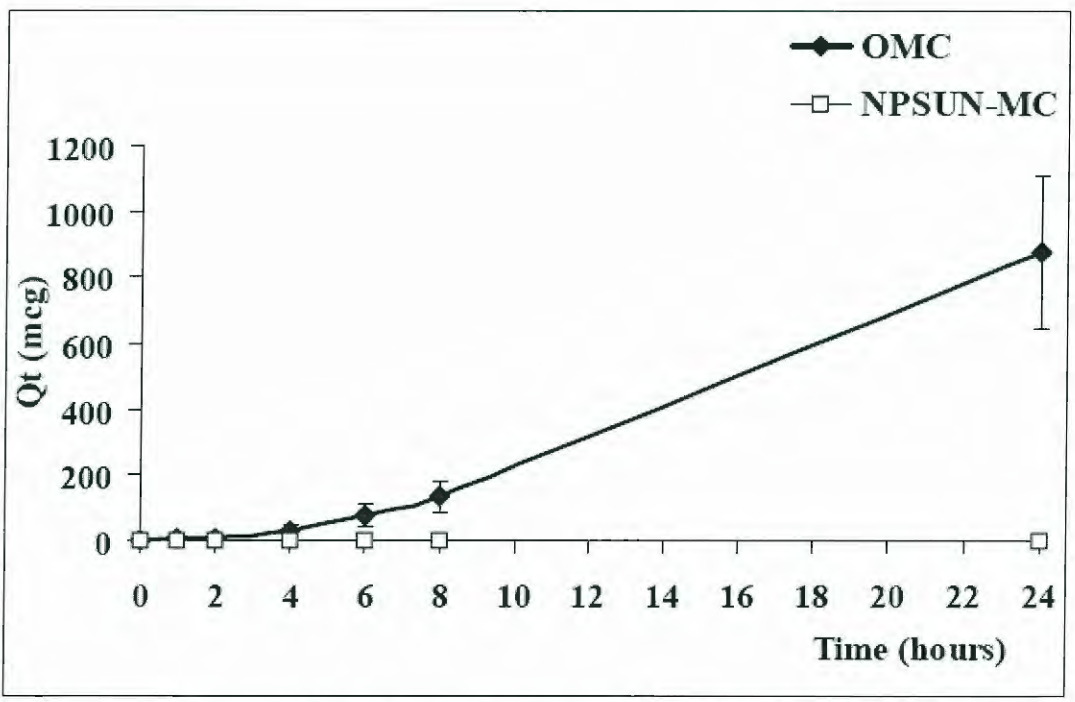

Fig. 2 Permeation profile of OMC and MC-NPSUN containing two UV absorbing units across nude mice skin following application of 10mg of the sunscreens on skin surface.

NPSUNs containing two or four methoxycinnamic UV absorbing groups (MC-NPSUN) were synthesized and characterized by various physico-chemical methods including ^{1}H-NMR, UV spectroscopy, mass spectroscopy, FTIR and elemental analysis. The results confirmed the structure of new sunscreen derivatives, showing that jojoba oil backbone was linked to UV absorbing methoxicinnamate units. It is noteworthy that MC-NPSUNs were found to possess a UV absorption spectrum similar to OMC and could be easily formulated in standard cosmeceutical and pharmaceutical topical products. Moreover, absolutely no permeation of MC-NPSUN across the skin was observed in 24 hours *in vitro* permeation experiments either following application of neat compounds (Figure 2) or after application of MC-NPSUNs formulated in o/w cream, in o/w cream or in jojoba oil. Another beneficial characteristic of NPSUNs is their high skin substantivity which could minimize the need for repetitive applications.

These results point toward a high applicability of new sunscreens based on jojoba oil with an advantage of skin non-penetrability. The non-penetrating characteristics of NPSUN new compounds are not only an important improvement over the currently used sunscreens but also are seen as meeting the future requirements of sunscreens. The use of NPSUNs will generate new improved cosmetic and pharmaceutical products as demanded by the market and health care needs.

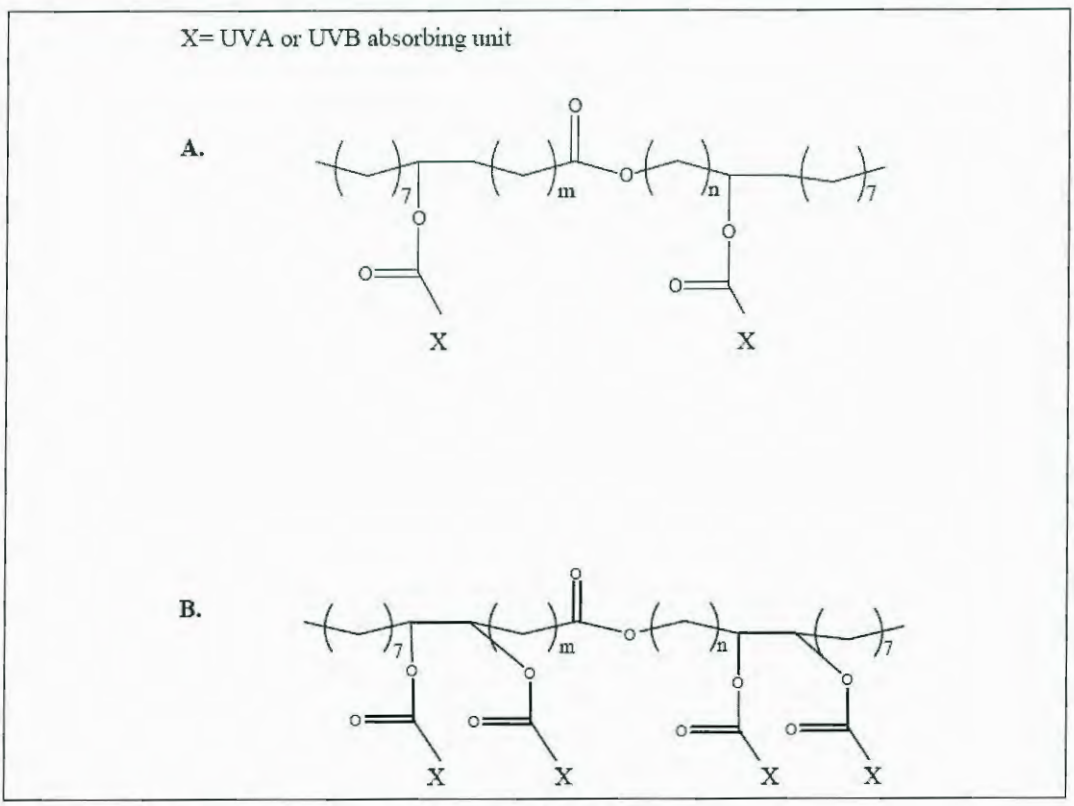

Fig. 3 Structure of NPSUN derivatives with two (A) and four (B) UV absorbing units.

Ethosomes for Efficient Intracellular and Dermal Delivery of Vitamin E to Prevent Damages Associated with UV Exposure

Previous studies have demonstrated that intracellular uptake of tocopherol is necessary for optimal photoprotection effect and prevention of malignant processes [9, 10].

For this reason we investigated Vitamin E skin and intracellular accumulation from a novel delivery carrier, ethosomes, aiming at enhanced penetration for inhibition of UV-induced photocarcinogenesis. Ethosomes are soft phospholipid vesicles containing ethanol tailored for enhanced delivery of active agents into the skin and across the cellular membranes [11, 12]. Due to their unique structure, ethosomes are able to encapsulate and deliver into the skin cells highly lipophilic molecules, such as alpha-tocopherol.

Tocopherol ethosomes were prepared and characterized for their physico-chemical characteristics [13]. Transmission electron (TE) microscopical visualization of alpha-tocopherol ethosomes was performed following negative staining with a 1% aqueous solution of phosphotungustic acid. TE micrographs of ethosomes containing alpha-tocopherol showed multilamellar vesicles with the bilayers extended to the core. The entrapment ability of ethosomes for vitamin E as determined by ultracentrifugation was 85-99% for systems possessing various compositions. This very good encapsulation ability could be explained by the high lamellarity of ethosomal vesicles as well as by the presence of ethanol in the system.

Skin and intracellular accumulation of alpha-tocopherol from ethosomes was further evaluated. In skin penetration experiments following 24 hours of non-occlusive application of alpha-tocopherol, 53.7±17.5% of the vitamin was accumulated in full thickness skin. In Confocal Laser Scanning Microscopy (CLSM) experiments cellular uptake of alpha-tocopherol ethosomes was followed by including rhodamine red labeled phospholipids (RR) in the lipid phase of the vesicles. CLS micrographs (Figure 4) following one hour incubation of 3T3 fibroblasts show that fluorescent phospholipid from ethosomes was evenly distributed throughout the cytoplasm. In contrast, when delivered from classic liposomes, the phospholipid was mainly seen on the surface of fibroblast and not within cells.

These results indicate that ethosomes efficiently delivered their contents into skin cells, while liposomes were attached to the cellular membrane with no cytoplasmatic uptake. In a following experiment, to quantify intracellular delivery of alpha-tocopherol into the skin cells, the vitamin was extracted from 3T3 dermal fibroblasts after one and four hours incubation with ethosomes and classic liposomes and assayed by HPLC with fluorescent detector. Intracellular concentrations of 731±189 and 1736±181 ng/mL alpha-tocopherol were measured following incubation of fibroblasts with ethosomes for one and four hours, respectively (Figure 5). Accumulation of alpha-tocopherol in fibroblasts after application from liposomes was significantly lower and not affected by the incubation time (470±23 and 508±44 ng/mL, respectively). These results correspond well with the CLSM data, showing that liposomes were attached to the cell surface and did not permeate into the cytoplasm [13]. The lack of toxicity of ethosomal carrier was evidenced from live/dead viability/cytotoxicity assay used to determine intracellular esterase activity and cell membrane integrity of 3T3 dermal fibroblasts following treatment with ethosomal system. These results are supported by data from a clinical study on human volunteers, which revealed that ethosomes are safe to skin and do not cause skin erythema [14].

The main targets of Vitamin E protective effect

are located in the intracellular compartments. Ethosomes caused efficient accumulation of alpha-tocopherol within the skin and dermal cells, pointing toward a high potential of this delivery system to enhance skin photoprotective effects of the antioxidant.

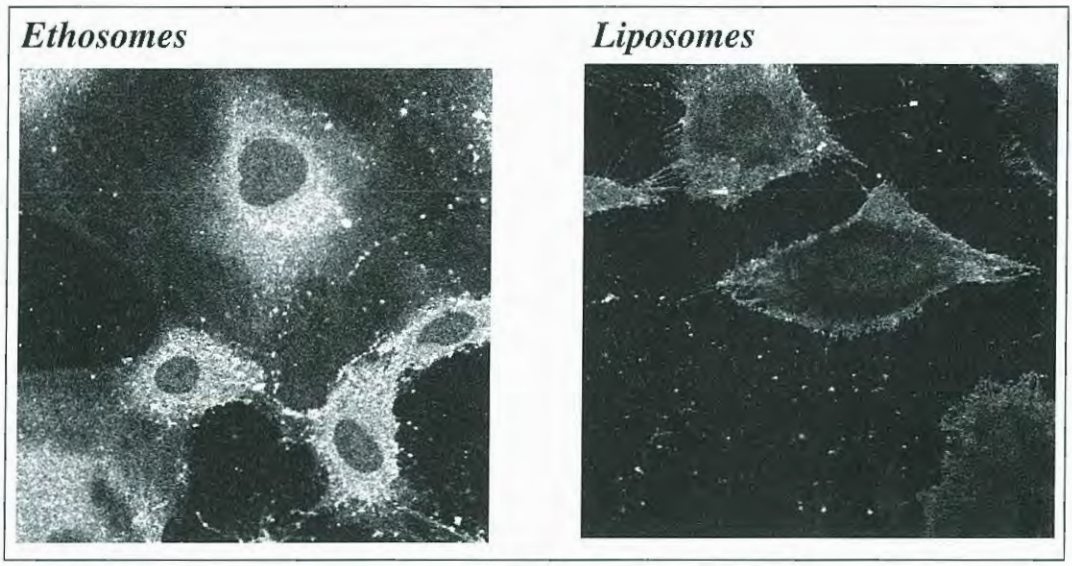

Fig. 4 CLS micrograph of rhodamin red labeled phospholipid (RR) intracellular accumulation from alpha-tocopherol ethosomes vs. liposomes.

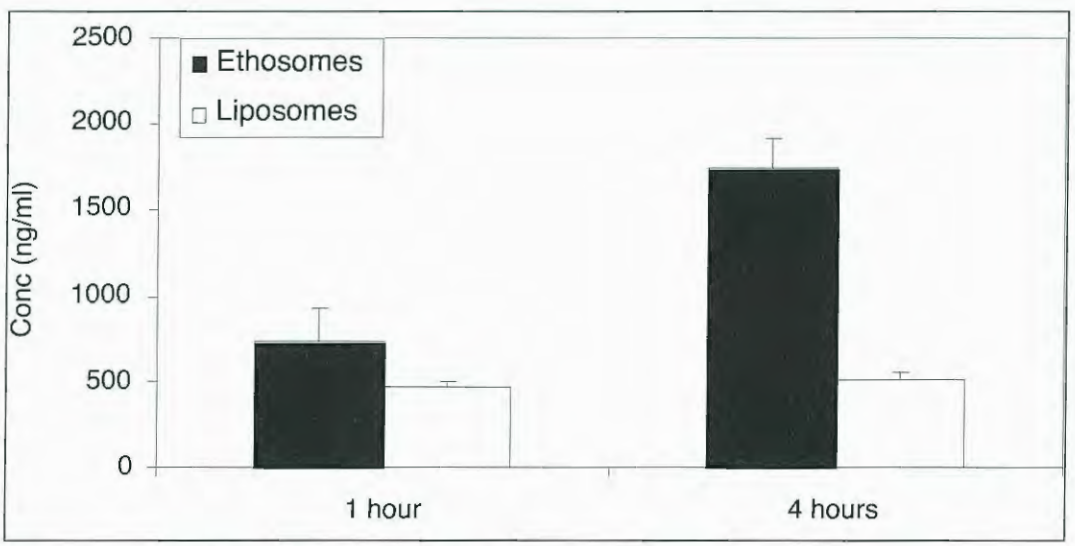

Fig. 5 Quantification of intracellular delivery of alpha-tocopherol from ethosomes vs. liposomes.

CONCLUSIONS

To summarize, this paper describes a combined approach for efficient protection from UV-induced photodamage: (1) use of NPSUNs, a new generation of chemical sunscreen derivatives of jojoba acting on the skin surface and lacking transdermal absorption, followed by (2) the use of ethosomes for enhanced skin and intracellular delivery of radical scavengers (Figure 1). Prevention of sunscreen systemic absorption will diminish the potential of sunprotectors to cause general side effects, while powerful delivery of alpha-tocopherol to the site of its action will increase its efficiency in inhibiting sun related carcinogenic processes. From a practical point of view, we propose the use of NPSUN based products during direct UV-exposure and the application of ethosomal alpha-tocopherol formulations after short or long term contact with solar radiation.

References

1) **Kraemer KH. (1997)** Sunlight and skin cancer: Another link revealed. *Proc. Natl. Acad. Sci. USA,* **94:** 11-14.
2) **Armstrong BK, Kricker A. (1993)** How much melanoma is caused by sun exposure? *Melanoma Res,* **3**: 395-401.
3) **Chatelain E, Gabard B, Surber C. (2003)** Skin penetration and sun protection factor of filters: effect of the vehicle. *Skin Pharmacol. Appl. Skin Physiol.,* **16:** 28-35.
4) **Gustavsson Gonzalez H, Farbort A, Larko O. (2002)** Percutaneous absorption of benzophenone-3, a common component of topical sunscreen. *Clin. Exp. Dermatol.,* **27:** 691-694.
5) **Schlumpf M, Cotton B, Conscience M, Haller V, Steinmann B, Lichtensteiger W. (2001)** *In vitro* and in vivo estrogenicity of UV screens. *Environ. Health Perspect,* **109:** 239-244.
6) **Schlumpf M, Schmid P, Durrer S, Conscience M, Maerkel K, Henseler M, Gruetter M, Herzog I, Reolon S, Ceccatelli R, Faass O, Stutz E, Jarry H, Wuttke W, Lichtensteiger W. (2004)** Endocrine activity and developmental toxicity of cosmetic UV filters--an update. *Toxicology,* **205:** 113-22.
7) **Touitou E, Bergelson L. (2002)** Products for preventing penetration into the skin. Patent pending.
8) **Miwa TK (1971)** Jojoba oil wax esters and derived fatty acids and alcohols. *J Am Oil Chem Soc* **48:** 299-306.
9) **McVean M, Liebler DC. (1999)** Prevention of DNA photodamage by vitamin E compounds and sunscreens: roles of ultraviolet absorbance and cellular uptake. *Mol Carcinog* **24:** 169-76.
10) **Trevithick JR, Xiong H, Lee S, Shum DT, Sanford SE, Karlik SJ, Norley C, Dilworth GR. (1992)** Topical tocopherol acetate reduces post-UVB, sunburn-associated erythema, edema, and skin sensitivity in hairless mice. *Arch. Biochem. Biophys.,* **296:** 575-82.
11) **Touitou E, Dayan N, Bergelson L, Godin B, Eliaz M (2000)** Ethosomes- novel vesicular carriers for enhanced delivery: characterization and skin penetration properties. *J. Control Release,* **65:** 403-418.
12) **Godin B, Touitou E (2004)** Mechanism of bacitracin permeation enhancement through the skin and cellular membranes from an ethosomal carrier. *J. Control Release,* **94:** 365-379.
13) **Lavy S (2002)** Ethosomes for enhancement of skin penetration of alpha-tocopherol, M.Sc. Thesis, The Hebrew University of Jerusalem, Jerusalem, Israel.
14) **Paolino D, Lucania G, Mardente D, Alhaique F, Fresta M (2005)** Ethosomes for skin delivery of ammonium glycyrrhizinate: *in vitro* percutaneous permeation through human skin and in vivo anti-inflammatory activity on human volunteers. *J. Control Release,* **106:** 99-110.

Author Address:

Prof. Elka Touitou
Department of Pharmaceutics
School of Pharmacy
Faculty of Medicine
The Hebrew University of Jerusalem
POB 12065, Jerusalem 91120, Israel
Fax: 972-2-6757611
e-mail: touitou@cc.huji.ac.il