Liposomes as a Stabilizing Carrier System for Vitamins

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Abstract

The benefits of vitamins in the cosmetics and dermatology are well known since years but getting a stable formulation is quite dependent on the derivatives used.

The encapsulation of vitamins into liposomes offers the formulator significant advantages in terms of stability of the vitamins, enhancing their properties as well as penetration of the actives into the epidermis.

Vitamin A:

Vitamin A promotes the enzyme activity of the skin and increases its collagen content, and is thus able to regenerate skin which has aged prematurely as a result of exposure to UV radiation. To a certain extent, vitamin A improves the skin's softness and smoothness, and brings about a significant increase in its elasticity. Latest findings show that vitamin A and its metabolites contribute to certain genes being expressed to a greater or smaller degree, and that processes responsible for the development of cancer are prevented (Prof. Dr. G. Jahreis, University of Jena).

Vitamins E and C:

Lipophilic vitamin E and water-soluble vitamin C represent an effective combination to act as antioxidants, free radical scavengers and peroxide neutralisers, in this way helping to prevent skin ageing as a result of UV radiation.

Vitamin E can help to reduce the formation of UV radiation-induced erythema, and if added to cosmetic formulations can increase the light protection factor [G. Erlemann and R. Merkle; SÖFW Journal 117 (10), 379-384, 1991].

Dermatologists recommend the use of vitamin E for the following: the inhibition of inflammatory processes, relief of itchiness, improvement of wound healing and scarring, as well as to support the treatment of acne [H. Möller et al.; Fett-Wissenschaft, Technologie 8, 295-315, 1989].

1. Penetration of ROVISOME ACE

The ability of the vitamins to permeate the human skin (200 μm thick viable skin) was determined by Nimbus GmbH, Leipzig, with

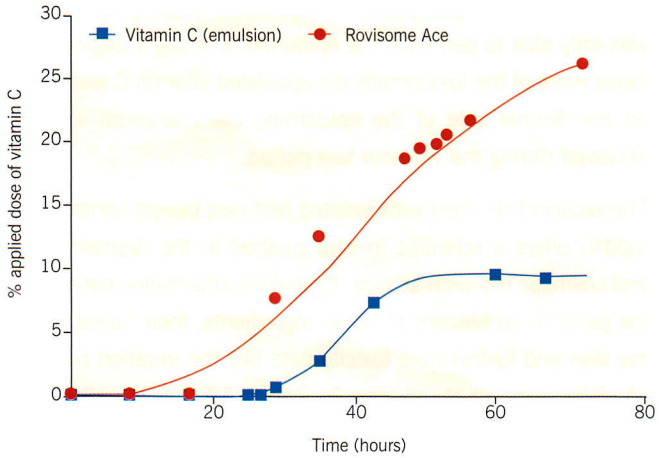

Figure 2. Penetration of Vitamin C (MAP) through the skin

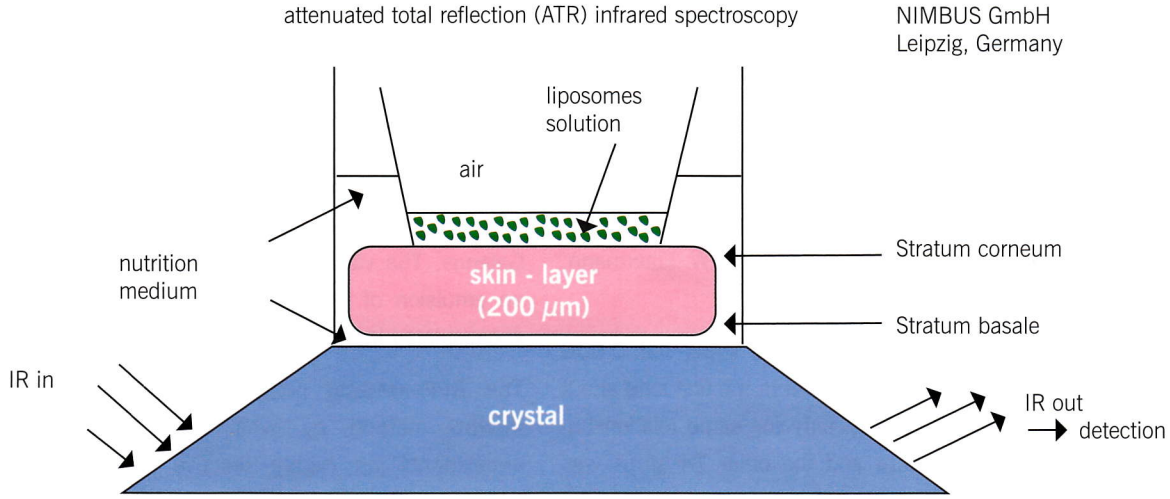

Figure 1. Set up of the penetration test via ATR

the help of a new method ATR-FTIR spectroscopy (Attenuated Total Reflection Fourier Transform Infrared Spectroscopy).

With this method, the epidermis of a human skin preparation is positioned on a crystal with the dermal side downwards, and is kept under physiological conditions for the 70-hour test period. Application of the substances to be examined, is followed by the measurement of the kinetics of the vitamins' permeation. The advantageous characteristics of this method guarantee that only the concentration of those substances is measured that have permeated the skin during the test period and can be detected in the physiological nutrient solution [A. Vierheilig and T. Braunschweig, Parfümerie und Kosmetik, 1-2, 20-21, 1998].

The penetration behaviour of the vitamins (vitamin E-acetate and magnesium ascorbyl phosphate) from an emulsion was determined and then compared with that of the liposomally encapsulated vitamins (ROVISOME ACE).

The emulsified water-soluble magnesium ascorbyl phosphate was only able to permeate the epidermis to a slight degree. Four times more of the liposomally encapsulated vitamin C was found on the dermal side of the epidermis, the maximum was not achieved during the 70-hour test period.

The excellent skin test with isolated perfused bovine udder (BUS-model) offers a scientific in-vitro method in the dermatological and cosmetic research as well. It provides information concerning the penetration kinetics of active ingredients, their interaction in the skin and furthermore toxicity data like the irritation potential [W. Pittermann et al., In Vitro Toxicology 10, 17-21, 1997; Th. Förster et al., J. Cosm. Sci., 147-157, 1999]. The skin of udder is very fine, moderately hairy and possesses an epidermis and metabolism which is comparable to human skin.

In three independent studies, vitamin E-acetate was applied in a lamellar creme or in the same formulation encapsulated in ROVISOMEs onto the skin of udder (3 – 4 g / 100 cm^2). After 30 minutes the skin was cleaned by paper towels and adhesive tape stripping were carried out (15 strips) as well as from untreated skin. The amount of vitamin E-acetate per cm^2 tape strip was measured by conventional HPLC analytics work [W. Pittermann, Parfümerie und Kosmetik 3, 38-41,1999].

The natural vitamin E-acetate content in 15 strips was 0.056 μg vitamin E-acetate / cm^2 tape strip. Just in the first strip about 50% of vitamin E can be found. Nearly the same relationship between the surface (first strip) and the other 14 strips was found after 30 minutes for the lamellar creme (42% / 58%) and also for the ROVISOME E-acetate formulation (44% / 56%). This means the same distribution pattern was determined for untreated and treated skin independent of the formulation.

But the total amount of vitamin E-acetate found in 15 strips of treated skin significantly differs.

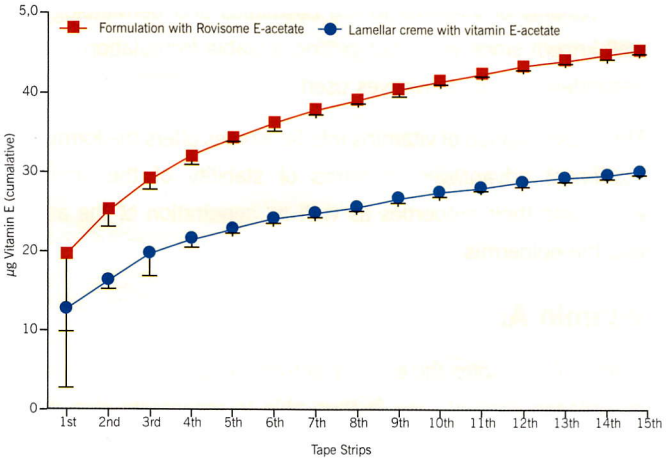

Figure 3. Penetration of Vitamin E (E-Acetate) into the skin

Liposomally encapsulated vitamin E-acetate was found to be 45.35 μg vitamin per cm^2 tape strip after 30 minutes compared with 30.04 μg per cm^2 tape strip for the lamellar creme.

This means ROVISOMEs are able to penetrate out from a formulation into the skin with carrying the active ingredients into deeper skin layers.

ROVISOMEs act as a carrier system which is capable of transporting the vitamins into the skin. The results corresponds to the findings of Röding and Artmann [J. Röding und C. Artmann, in Liposome Dermatics, Springer Verlag Berlin, Heidelberg, 1992]. Once in the skin (stratum corneum), the active ingredients are enzymatically released from the liposomes and are then free to penetrate even deeper.

2. The radical scavenging properties of ROVISOME ACE

The radical scavenging properties of the vitamins A, C, and E were measured in a study carried out by the Berlin company Galenus. The vitamins were available both in their free form, an emulsion of vitamins, as well as in their protected form, encapsulated in liposomes (ROVISOME ACE).

The RPF (radical protection factor) is measured by an in-vitro method by which the reducing properties of antioxidants on highly reactive semi-stable free radicals are determined.

A defined quantity of each sample is dissolved in a mixture of water and alcohol, and then mixed with the test standard containing the free radicals. The impaired electrons of these radicals can be detected by ESR (Electron Spin Resonance). The intensity of the ESR signals before and after mixing the samples with the test standard are used to quantify the concentration of non-reduced free radicals and to calculate the RPF (10^{14} free radicals / mg of substance utilised) [T. Herrling et al., Conference Proceedings, IN-COSMETICS, Düsseldorf, Mai 1997].

It can be seen that, with a RPF of 9.6, the radical scavenging properties of the vitamins in their liposomal form are four times greater than those of the non-encapsulated vitamins in the emulsion (RPF 2.4).

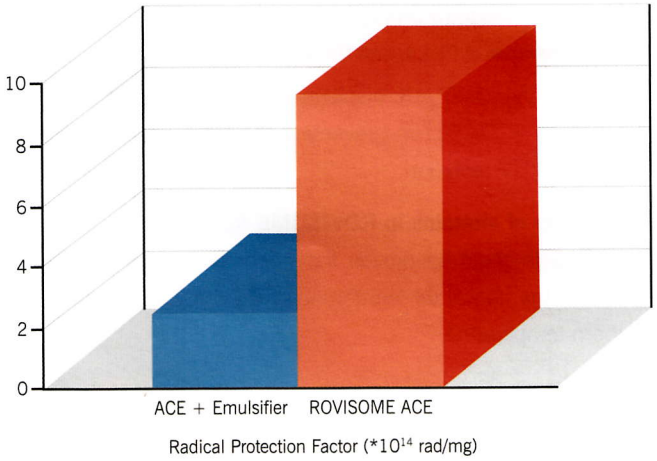

Figure 4. Radical protection factor of vitamins

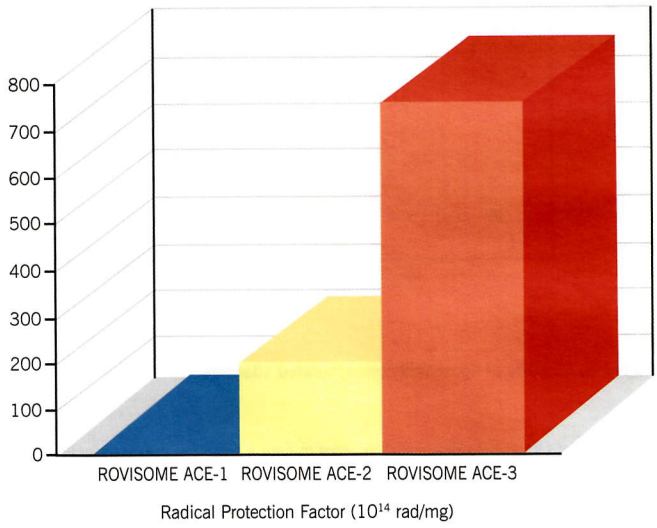

Figure 5. Radical protection factor of liposomally encapsulated vitamins

Since both preparations have the same vitamin content, it follows from the difference in activity that the membrane protects the liposomally encapsulated vitamins. It is assumed that the physical arrangement of the vitamins in relation to one another, which corresponds to the natural formation within the cells, prevents premature oxidation.

Improvements to the vitamin combination in ROVISOME ACE lead to increased scavenging properties. ROVISOME ACE-II contains pure tocopherol instead of vitamin E-acetate, and in ROVISOME ACE-III the magnesium ascorbyl phosphate has been replaced by vitamin C-palmitate.

In the case of ROVISOME ACE-II, it was possible to increase the RPF by the factor 20 ($188*10^{14}$ free radicals / mg), and with ROVISOME ACE-III, an RPF of $750*10^{14}$ free radicals per milligram of liposomes suspension utilised was achieved.

Our data gives clear evidence that the stability, and consequently the activity, of the vitamins are raised when they are encapsulated in the lipid membrane, i.e. liposomes. Studies carried out by I. Arsic and S. Vidovic also indicate the increased stability of liposomally encapsulated vitamin A-palmitate in a formulation subjected to UV radiation [I. Arsic and S.Vidovic, Conference Proceedings, Int. Conference of IFSCC, Budapest, April 1997].

3. Protective function of the vitamins against UV-related DNA damage

As a part of her diploma thesis, Iris Strodtholz examined, with the help of the Comet assay, the effect of UV-A radiation on the DNA of keratinocytes and the protective effect of antioxidants [Iris Strodtholz, Diploma thesis, Fachhochschule Lippe, November 1997].

The Comet assay is a single-cell electrophoresis by which it is possible to determine the extent of DNA damages. Keratinocytes (HaCaT cells) are incubated for 24 hours with the antioxidants to be examined (vitamins, or ROVISOME ACE). After 10^5 cells have been embedded in an agar gel, the cells are exposed to UV-A light (5 J/cm^2) (SOL 500 sun simulator). The keratinocytes are then lyzed under alkaline conditions, so that only the nucleus of the cell remains. During the following electrophoresis at pH 13, the negatively charged single-strand fragments and partially unwound DNA migrate towards the anode.

After dying with ethidium bromide and depending on the DNA breaks, it is possible to observe an increase in the DNA that has migrated from the nucleus of the cell (comet tail) under a fluorescence microscope, whereas the intact cell nucleus can be seen as a single spot in case of the undamaged cell.

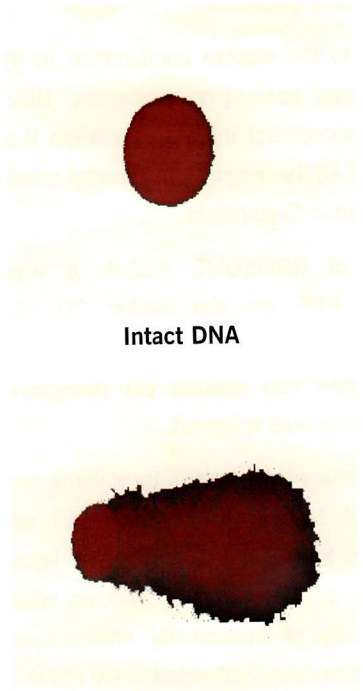

Figure 6. Comet Assay

In the Comet assay, the protective effects of ROVISOME ACE (application: 0.001 %, this means a vitamin content of 0.5 μg/ml) against UV-A related damages to the DNA in keratinocytes was tested, and compared with unfilled liposomes (0.001 %). In a further test, pure tocopherol or magnesium ascorbyl phosphate (3 mM of each) was used.

Whereas the untreated cells displayed a high level of DNA migration (synonymous with damage), incubation with even the unfilled liposomes (polyunsaturated fatty acids) enabled the extent of any damage done to be reduced by the factor 1.3.

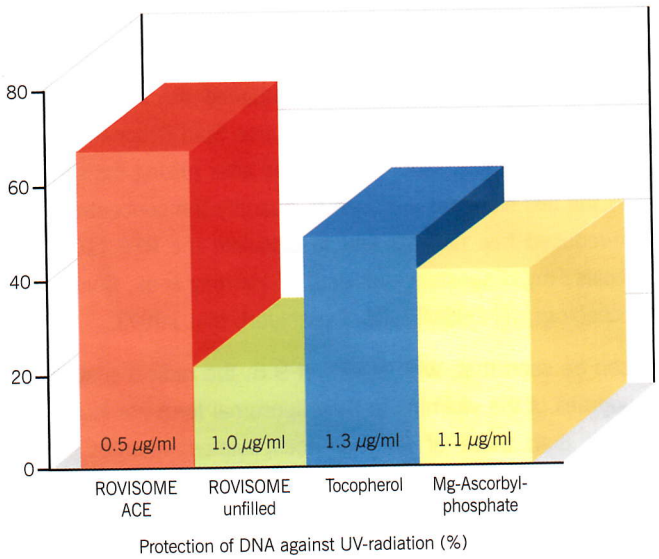

Figure 7. Protection of DNA against UV-radiation by vitamins

Only the ROVISOME ACE displays a highly significant protective function ($p < 0.001$) in preventing the DNA against damages induced by UV radiation.

4. Stability of vitamins in ROVISOME ACE

Via HPLC is could be proven that the vitamins are stable over weeks in the liposomal membrane of ROVISOMES when stored at room temperature.

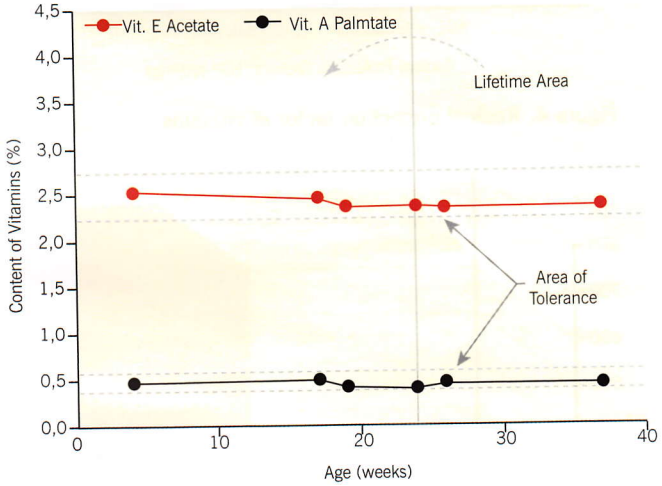

Figure 8. Stability of liposomally encapsulated vitamins

Authors Biography:

Dr. Gabriele Blume

Born 09.01.1955. After finishing grammar school in 1974 Dr. Blume completed an apprenticeship to become a biological engineer at the Hoechst AG in collaboration with the technical University of Gießen (Germany).

Afterwards she studied biology and biotechnology at the University of Essen where she graduated from in 1985. Subsequently, she continued with Ph D studies at the technical University of Munich, finishing her dissertation in 1991 with the subject "Systemically applied liposomes in the medicine". Then she moved to the University of Utrecht (Netherlands) in order to take a post-doc position dealing with the "chemical modification of liposomes to enhance specific targeting". In 1992 she started working for IDEA GmbH (Munich) as Manager R&D, which means the development and characterisation of topically applied liposomes (Transfersomes) for the pharmaceutical industry. After switching to ROVI Cosmetics in 1996, she has been responsible as Vice President R&D for the research and development of novel drug carrier systems and liposomes for the dermal application.. She collaborates effectively with universities in Germany (Marburg, Saarbrücken, Jena) with specific research tasks to clarify the properties and efficacy of liposomes loaded with active ingredients.