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Structural and Functional Quality Control of Cosmetic Products by ESR Spectroscopy

Keywords:

Summary

ESR (Electron Spin Resonance) Spectroscopy is a powerful tool for both structural and functional analyses on cosmetic products or ingredients. Considering as example the quality control of a cosmetic formulation containing Q10 encapsulated into special liposomes (Rovisomes®), the *in vitro* and *ex vivo* activity and the penetration of Q10 into human skin biopsies was analysed by ESR Spectroscopy. The activity of antioxidants strongly depends on their chemical environment and on the interaction with other components of the cosmetic formulation. Liposomes facilitate the penetration of the encapsulated substances into the skin and can act as »activators« for an antioxidant. The stability of liposomes in a cosmetic formulation is therefore an important structural quality control. The *in vitro* Antioxidative Power (AP) of Q10 was analysed by ESR spin probing techniques and was found to be higher in the liposomal encapsulated form compared with the pure Q10. The penetration ability into human skin biopsies was significantly higher when Q10 was applied in the encapsulated form and the Antiradical Power (ARP) was correspondingly increased.

Introduction

The increasing complexity of the composition of cosmetic products requires new methods for the quality control of the ingredients as well as the activity of the final product. Beside the basic ingredients of the formulations, cosmetic products are enriched with active substances such as vitamins, coenzymes, UV-filters. Most of these active substances are antioxidants or radical scavengers and are designed to enhance the total Antioxidative Power (AP) or the Antiradical Power (ARP) of the formulation and of the skin.

The activity of these additives is strongly influenced by the chemical environment in the formulation and can change by changing the chemical-physical properties of the probe (temperature, acidity, lipophilic/hydrophilic character). If more than one active substance is present, they can interact to give synergistic or antisynergistic effects.

Moreover, these active substances are often encapsulated in carrier systems like liposomes or silicones. The stability of those carrier systems in a complex cosmetic formulation is an important criterion and requires a non banal characterisation. The interaction of the encapsulated active substance with the carrier system can lead to an higher or lower activity of the antioxidant/radical scavenger.

The aim of a cosmetic formulation is to bring the active substances into the skin, thus to enhance the penetration through the stratum corneum. The measurement of penetration and activity *in vivo* can be considered the final quality control.

All of the above structural and functional quality controls can be performed by one single technique: the ESR spectroscopy.

The ESR spectroscopy is based on the absorption of microwave frequencies by a system containing unpaired electrons which is orientated in a magnetic field. It is therefore the only technique able to detect directly free radicals. Moreover this technique is independent on the chemical-physical properties of the probe, i.e. turbidity, opacity, viscosity. It is possible to measure directly different cosmetic formulations as crèmes, lotions, gels without previous dilution or extraction. Different testing protocols allows to study both functional and structural properties **(Table 1)**.

Herein we want to present some examples of structural and functional quality control by ESR spectroscopy. The complex system under study was a liposome-Q10 crème where the active substance, the coenzyme Q10, was encapsulated into special liposomes (Rovisomes®) and these carrier systems were added to a crème basis. First the stability of the li-

posomes inside the oil-in-water emulsion had to be tested. We will present the analysis of liposomes in various environments and the influence of emusifiers, that are normally added to cosmetic crème formulations on the stability of liposomes. Further the interaction of the Q10 with the liposomal membrane was investigated and the influences of these interactions on the Antioxidative Power of the Q10 – liposomes in crèmes are discussed. Finally the penetration ability and *in vivo* activity on human skin biopsies is presented.

		ESR method
Functional quality control	**Antioxidative Power (AP)**	Reduction of stable test radicals
	Antiradical Power (ARP)	Induction of free radicals and spin trapping
	Penetration	Spin trapping / reduction of stable test radicals
	in vivo activity	Spin trapping / reduction of stable test radicals
Structural quality control	**Stability of carrier systems**	Spin labeling
	Interaction of active species and carriers	Spin labelling and reduction of stable test radicals

Table 1 Functional and structural quality controls by ESR spectroscopy.

Structural quality control: Stability of liposomes

Liposomes are small lipid vesicular carrier systems that present a intern hydrophilic core surrounded by lipid bilayer membranes. In the water core or inside the membrane active substances can be encapsulated. The liposomes (Rovisomes®) used in the present study were SUV (small unilamellar vesicles), consisting in one single bilayer membrane of phosphatidylcholine (PC) with a diameter of approx. 100 nm. The membrane was labeled with an ESR-active nitroxide spin label, an stearic acid derivative (5-DSA). This spin label gives an ESR spectrum that allows to define the order parameter, mobility, homogeneity and rigidity of the labeled membrane **(Fig. 1)**.

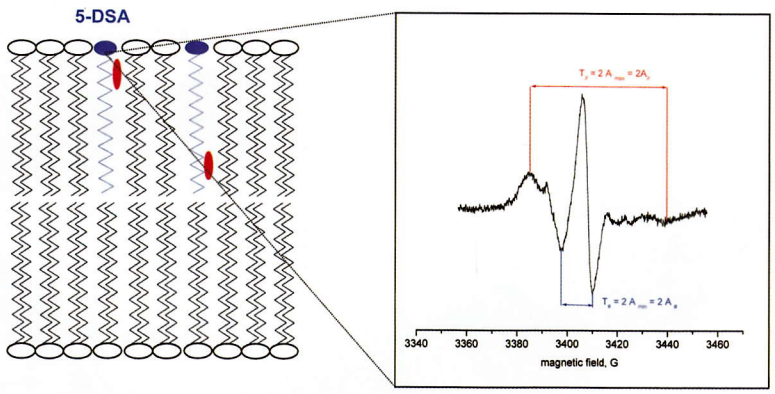

Fig. 1 Schematic representation of a model membrane labelled with 5-DSA and its correspondent ESR spectrum.

If the integrity of the liposomal membrane is altered or the lipsomes are disrupted, the ESR spectrum changes. Liposomes in an pH neutral buffer solution (PBS) can be disrupted by the addition of a strong surfactant, like Triton X.

Fig. 2 shows the spectra of 5-DSA labeled liposomes in phosphate buffer alone (black line), with 5% ethanol (red line), and with 5% Triton X (green line). From the change in the spectral manifold it results that only Triton X and not ethanol is able to disrupt the liposomal membrane.

The presence of tensioactive emulsifiers inside a cosmetic formulation can lead to a minor stability of liposomes. **Fig. 3** shows four different emulsifiers (5% w/w) were tested on the liposomal stability. The relative changes of the order parameter S was analysed from the spec-

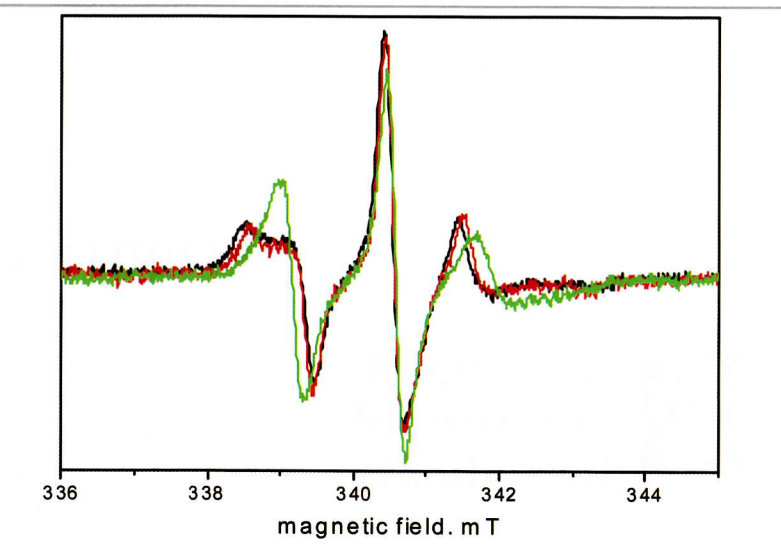

Fig. 2 ESR spectra of D-DSA labelled Rovisomes in PBS (black line), 5% ethanol (red line), and 5% Triton X (green line).

tra. Emulsifiers E2 and E3 dramatically lowered the order parameter; the liposomal membrane is »softened«, the lipid mobility increases and the stability is compromised.
These examples of stability control performed by ESR spectroscopy elucidate the widespread possibilities to study the morphological features of carrier systems offered by this method. With respect to other analytical methods (i.e. chromatography, fluorescence), with the ESR spectroscopy the investigation of very different cosmetic formulations with different opacity and viscosity is possible.

Functional quality control: activity of Q10

Ubiquinones are one of the most important coenzymes, responsible for a widespread range of biological important activities. They are membrane-associated lipophilic antioxidants, that inhibit lipid peroxidation and regenerate tocopherol. The most abundant ubiquinone in human skin is Q10 **(Fig. 4)** and an enrichment of Q10 inside the skin is the main reason for cosmetic Q10 formulations.
Due to the liposomal encapsulation of Q10, the penetration into the skin is facilitated. But liposomes not only serve as an efficient carrier system, they can also activate the antioxidant capacity of Q10. The oxidated ubiquinone, present in cosmetic formulations has to be activated via reduction to the ubiquinol form with a high AP, and this activation can take place in an lipid environment such as the liposomal membrane. ESR structural analysis confirms the presence of Q10 inside the liposomal bilayer (data not shown).
The ESR spin probing technique allowed to measure the AP of the pure Q10 (oxidated form), the liposomal encapsulated Q10 and a mixed system containing empty liposomes and Q10 **(Fig. 5)**.
Different stable test radicals (NTX) were used to follow the reduction kinetics of the three systems. The pure Q10 was not redox-active (yellow symbols), the Q10 added to empty liposomes (blue symbols) showed a lag time followed by fast reductive activity and the liposomal en-

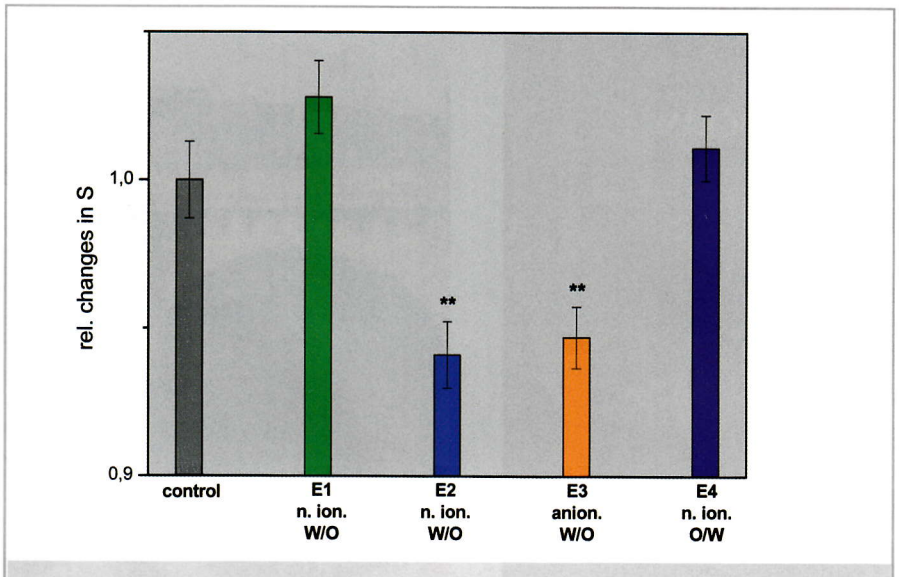

Fig. 3 Relative changes of the order parameter S calculated from spectra of 5-DSA labeled Rovisomes in the presence of different emulsifiers.

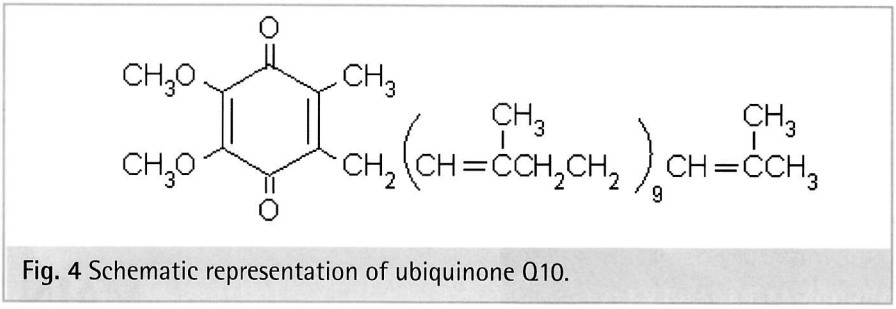

Fig. 4 Schematic representation of ubiquinone Q10.

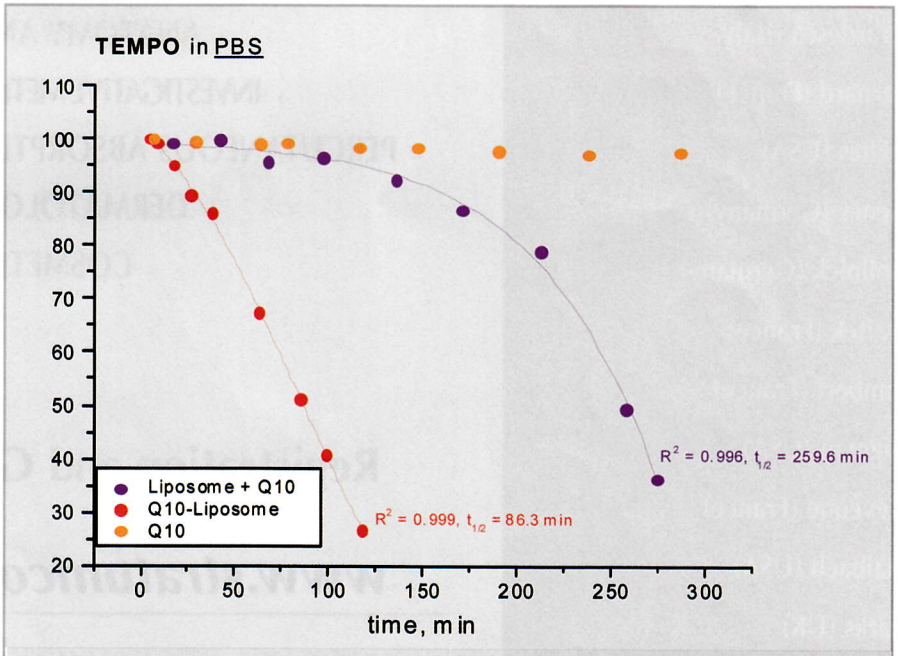

Fig. 5 Reduction kinetics of the nitroxyl (NTX) test radical TEMPO (2,2,6,6, tetramethylpiperidine 1-oxyl) in 5% w/v pure Q10 (yellow symbols), in 0.5% Q10 added to empty liposomes (blue symbols), and in 0.5% Q10-Rovisomes.

capsulated Q10 (red symbols) reduced the test radical immediately with a very fast kinetic.
From these kinetics, the half life time of the test radical was calculated and the inverse of t1/2 of the test radical in the three systems is shown in **Fig. 6**. The experiment has been performed both in buffer and in a crème formulation.
The higher is the 1/t 1/2 value, the higher is the antioxidative power of Q10.
In both systems, PBS and creme emulsion, the Q10-liposomes showed a significant higher activity as compared to the pure Q10 and the liposome + Q10.

Functional quality control: ex vivo activity

The penetration ability and *in vivo* activity of a cosmetic formulation is, of course, of primary interest and can be investigated with the same ESR technique. For the measurement on skin biopsies the skin is labeled with test radicals or spin trapping agents and the cosmetic formulation is applied on the skin surface (the statum corneum). If the active substances penetrate inside the epidermis, they will react with the ESR active labels. From one measurement it is, in principle, possible to obtain information about the penetration capacity (depth and velocity) as well as about the redox activity (AP) or radical scavenger activity (ARP) of an antioxidant. With this technique it is furthermore possible to determine the AP of the skin itself and the various factors that influences the AP (hydration, stress factors, UV irradiation, nutrition, use of cosmetics).
For the example chosen for the present work, the liposomal encapsulated Q10, the penetration and activity of the free ubiquinone versus the liposomal encapsulated one was to be determined.
The experimental design is shown in **Fig. 7**.
The skin biopsy is applied with the dermal side to a reservoir (filter paper) containing the spin trap PBN (alpha-phenyl-N-tert-butyl nitrone). PBN is able to trap oxygen free radicals (ROS) such as hydroxylradicals and superoxide anions and the PBN-adduct is detectable by ESR spectroscopy. The PBN diffuses from the

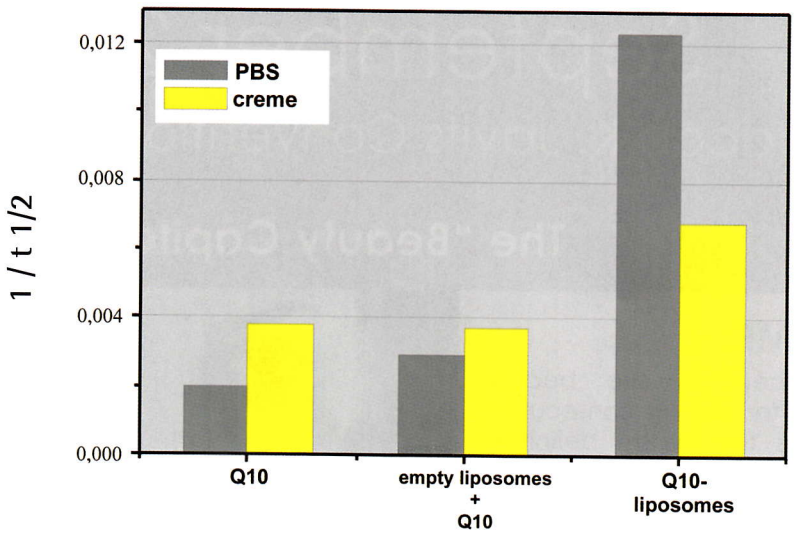

Fig. 6 1/t 1/2 values of the three Q10 formulatios (see Fig. 5) in PBS buffer (grey) and in crème (yellow).

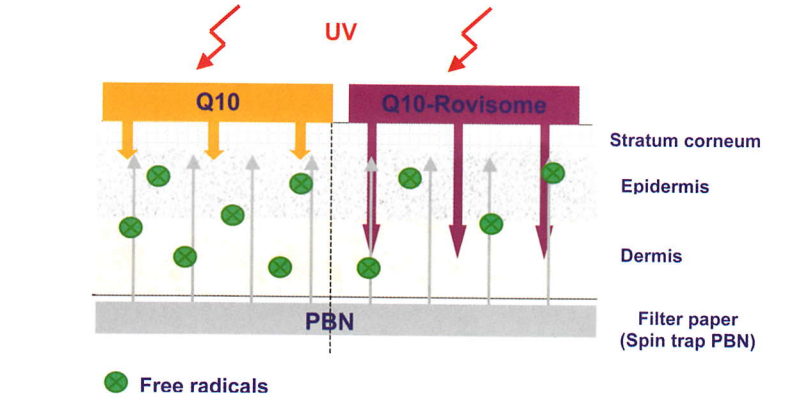

Fig. 7 Schematic representation of the experimental design of *ex vivo* ESR on human skin biopsies.

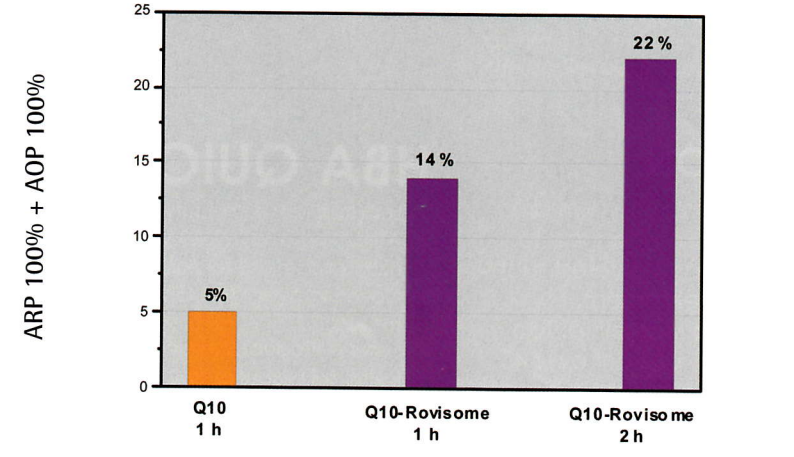

Fig. 8 Antiradical Power (ARP) of Q10 formulations in skin biopsies.

dermis up to the lower epidermis, but not to the stratum corneum. On the skin surface (stratum corneum) the pure Q10 in ethanol or the liposomal encapsulated Q10 is applied. Free radicals are induced by UV- irradiation of the skin. If Q10 penetrates into the epidermis, it will neutralize the induced free radicals, otherwise the PBN will trap them to give rise to an ESR signal. The results are shown in **Fig. 8**.

Due to the application of Q10, the AP of the skin biopsies was increased by 5% when Q10 alone was applied. The application of liposomal encapsulated Q10, although in a 10 times lower concentration, led to an 14% increase; when the preparation was applied for 2 hours the increase was up to 22%.

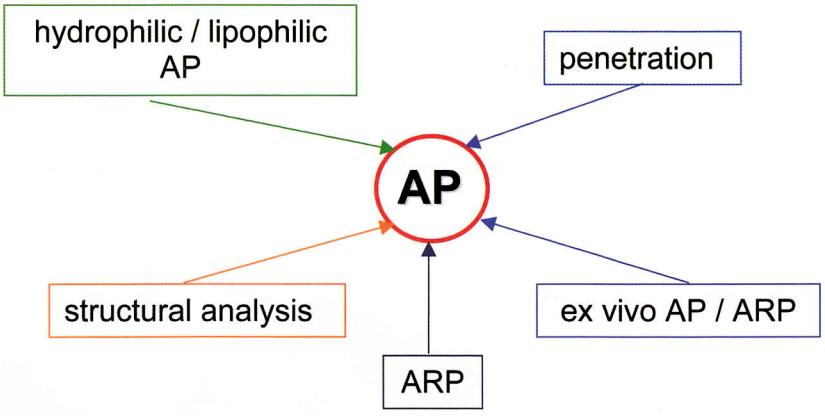

Fig. 9 Scheme for the determination of the Antioxidative Power, AP

Conclusion

The structural and functional quality control of the liposomal encapsulated Q10 is one example of a multitude of possibilities offered by ESR spectroscopy **(Fig. 9)**.

With one and the same technique it is possible to study the activity of redox-active ingredients of cosmetics, independent from the physical-chemical properties of the probes. Viscosity , opacity, lipophility and other characteristics are no longer disturbing factors for analytical studies. The structural analysis of carrier systems helps to find the best condition and to choose the right composition of the cosmetic formulation in order to stabilize and activate the active species. In our example, the liposomal encapsulation facilitate the penetration ability of Q10 and increase the Antioxidant Power (AP) of the ubiquinone. The antioxidative Power of an antioxidant can be differentiated into a hydrophilic or lipophilic AP. This differentiation could be of particular interest when more than one antioxidant is present and their activity is to be distinguished. The Antiradical Power (ARP) gives information about the protection from ROS (reactive oxygen species, free radicals) offered by a certain formulation (i.e. vitamins, UV filters). This method can be applied both in vitro and *ex vivo* in skin biopsies. The penetration of the active species into the skin can be analysed by ESR as well and it is, in most cases, the necessary condition for the *in vivo* activity.

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