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Preparation and Properties of Coenzyme Q10 Nanoemulsions

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Abstract

Coenzyme Q10 (CoQ10), also known as ubiquinone, is used for energy production within cells and acts as an anti-oxidant. Due to this dual function CoQ10 finds its application in different commercial branches such as drugs, food supplements, or cosmetics.

Since CoQ10 is highly lipophilic, the topical and oral bioavailability is very low. Several attempts have been made to improve absorption. Latest technical developments reveal that encapsulation of CoQ10 in nanoemulsions results in a significantly enhanced bioavailability. In addition, multiple nanoemulsions prepared according to a patented process even allow the administration of several incompatible substances at the same time.

This article gives an overview of current key developments of the encapsulation of CoQ10 in nanoemulsions. It highlights how encapsulation upgrades the bioavailability of CoQ10 and with this the efficacy of CoQ10. In addition, this article presents latest in vitro tests demonstrating the influence of CoQ10 on the synthesis of collagen I and on the activity of mitochondria and their resistance against stress of dermal fibroblasts and keratinocytes, respectively.

Introduction

Everyone requires energy to live. This energy is produced by combustion of carbohydrates or fats with oxygen. However, the use of oxygen will always also generate reactive oxygen species (ROS) which will damage the cells and therefore reduce the activity of cells. This will cause a general ageing process of cells and the whole body. Thanks to a compound named coenzyme Q10 (CoQ10) the human body possesses a pivotal player in energy synthesis. In mitochondria CoQ10 helps to build up adenosine triphosphate (ATP), the body's major form of stored energy. A second task of CoQ10 is the activity as an essential regenerating antioxidant scavenging free radicals such as ROS.

Since every cell consumes energy and needs antioxidant protection, CoQ10 is present in all cellular membranes of every single cell of the body. Caused by this ubiquitous presence in the body, as well as in the rest of nature, CoQ10 is also known as ubiquinone. However, deficiencies of CoQ10 in the human body have been reported to occur frequently. In addition, CoQ10 levels decline rapidly under stress or with advancing age. In case of deficiency CoQ10 has to be supplemented to guarantee the body's energy production and its essential antioxidant protection. (1,2)

Use of CoQ10 as a drug, food supplement and cosmetic ingredient

Although CoQ10 can be synthesized in the human body, it can happen that the body's synthetic capacity is not sufficient to meet the required amount of CoQ10. Cases of deficiencies of CoQ10 are reported in a variety of diseases, e.g. cardiovascular disorders. A randomized, double-blind clinical trial assessing 49 patients who experienced cardiac arrest (heart attack or accident), revealed that after an immediate treatment with a CoQ10 nanoemulsion such as described in this paper the survival rate increased more than 100 % versus placebo after 90 days. (3) Beside these life saving properties, CoQ10 also shows positive effects in migraine and Parkinson treatments. Latest clinical research resulted in an excellent positive effect on attack frequencies, headache days and days with nausea in migraine patients. (4) In different research programs for Parkinson's disease the efficacy of CoQ10 is now under investigation. Further interest in CoQ10 application was reported for gastric ulcer, muscle dystrophy, allergy and even cancer or AIDS. (1)

CoQ10 found its uses not only as a remedy but also as a food supplement and a cosmetic. Since the synthesis of CoQ10 in the body weakens in correlation with advancing age, daily dietary supplementation provides the required compensation for energy

and health. Additionally, poor nutritional habits or mental and physical stress may render CoQ10 supplementation advisable as well. (5) To give an example, the integrity of mitochondria is essential for the health of cells and organs. Mitochondrial DNA repeatedly undergoes mutations caused by oxidative stress. In comparison to nuclear DNA having a SOS DNA repair system, the mitochondrial DNA has no effective repair system to fight such mutations. Therefore, all mutations will accumulate during time and reduce the vitality of the cell. Hence, CoQ10 is an essential antioxidant for mitochondria to protect the integrity of its own DNA as good as possible. (6)

As a cosmetic ingredient CoQ10 mainly acts as an antioxidant to protect the cells against the ageing process induced by free radicals. Oxidative stress caused by free radicals or UV-irradiation plays a significant role in skin ageing. UV radiation is known to induce the formation of reactive oxygen species which are implicated as toxic intermediates in the development of photo-ageing. (7) The antioxidant activity of CoQ10 prevents untimely skin ageing and photo-ageing by enhancing the resistance of the skin and scavenging radicals. (8,9) A German research group found that CoQ10 also suppresses collagenase, an enzyme which causes damage of the connective tissue of the skin. The group additionally showed that CoQ10 is effective against UV mediated oxidative stress. (10) Taken together, these findings turn CoQ10 into a unique cosmetic substance which protects the skin from early ageing, wrinkle formation and loss of cell activity.

Chemistry and bioavailability of Q10, and the principle of nanoemulsions

CoQ10 belongs to the group of quinones. It is composed of a p-benzoquinone ring system and a polyisoprenoid side-chain. The length of the side-chain is responsible for the lipophilicity of the molecule. The side-chain in CoQ10 consists of ten isoprene units. This makes the molecule highly lipophilic. (11) Therefore CoQ10 can freely move within the cellular membranes. Unfortunately, the bioavailability of CoQ10 is very low in the intestines after oral application. (12)

Several attempts have been made in the last few years to improve the intestinal absorption of CoQ10. Researchers either tried to modify the molecular structure or change the compositions of the CoQ10 preparations to improve the bioavailability. As a matter of fact, the investigation of both structure modifications and delivery systems revealed that bioavailability of CoQ10 after oral application can be significantly enhanced choosing a proper formulation. (13,14)

Modern research now shows that CoQ10 nanoemulsions strikingly improve the bioavailability of the substance after oral application. (15) Daily application of 300 mg of powdered CoQ10 results only in a serum concentration of 1.8 μg/ml after 16 months. Thanks to encapsulation into nanoemulsions, the same daily dosage of CoQ10 enhanced serum concentration up to 5.2 μg/ml after only 6 weeks. (16,17)

These nanoemulsions improve dermal bioavailability as well. It is known that encapsulation of drugs in nanoemulsions and liposomes enhance the drugs' concentrations in the dermis compared to conventional formulations. (18) **Figure 1** shows the penetration of nanoparticles (nanoemulsion droplets) into the skin and the release of the encapsulated material (CoQ10) (Figure 1).

Features of nanoemulsions

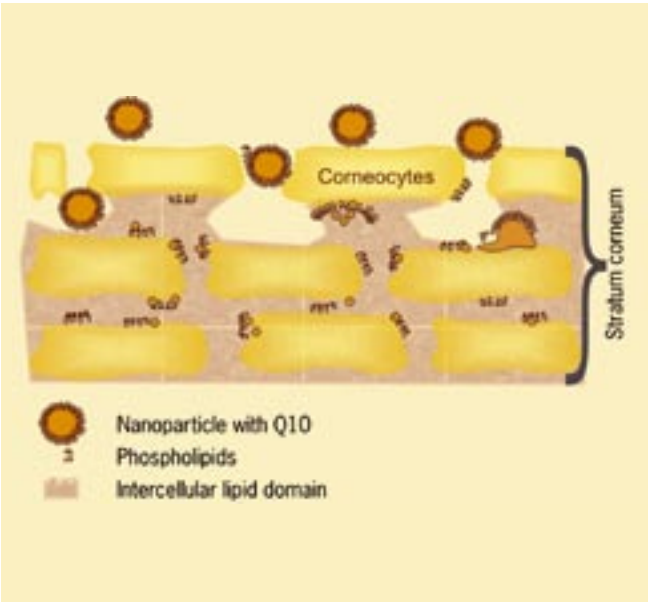

Figure 1:
Schematic illustration of the penetration of nanoemulsion droplets into the stratum corneum of the skin. After the release of the encapsulated material the substances can penetrate into deeper layers of the skin.

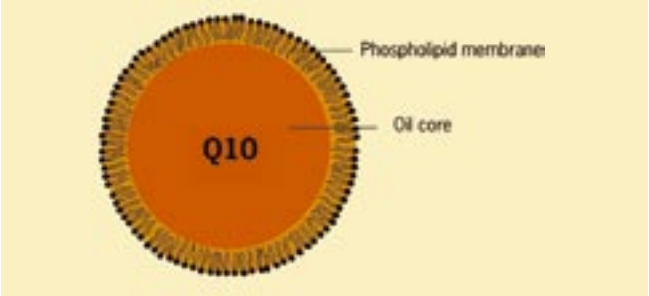

Figure 2:
Structure of a nanoemulsion droplet. Phospholipids are arranged according their lipophilicity in the border area of the liquid oil droplet

Nanoemulsions (also named nanoparticles) are oil-in-water emulsions having a small droplet size (30 – 300 nm). **Figure 2** illustrates the structure of a nanoemulsion droplet (figure 2).

Phospholipids build the border area of the droplet and separate the oily phase from the aqueous phase. **Figure 3** shows an electron microscope picture of a nanoemulsion containing CoQ10 (figure 3). The oil droplets containing CoQ10 are dispersed in the water phase and have a diameter of about 50 nm. The small size of the droplets is achieved through high pressure homogenization. (19) A notable advantage of a nanoemulsion is its outstanding stability, even at high temperatures up to 120 °C.

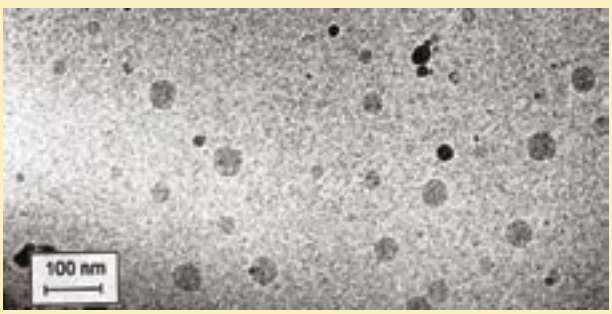

Figure 3:
Particle size of Q10 nanoparticles (around 50 nm) visualized by a TEM (transmission electron microscope).

Of special interest is also the preparation of transparent CoQ10 nanoemulsions. These preparations can be obtained if a droplet size of less than 60 nm can be achieved. This small droplet size will no longer scatter the light. However, CoQ10 will still absorb light and therefore this preparation looks orange (figure 4).

Figure 4:
Nanoemulsions containing CoQ10. 1: 7% CoQ10, 2: 0.7% CoQ10, 3: 0.07% CoQ10. The droplet size of all preparations is around 50 nm.

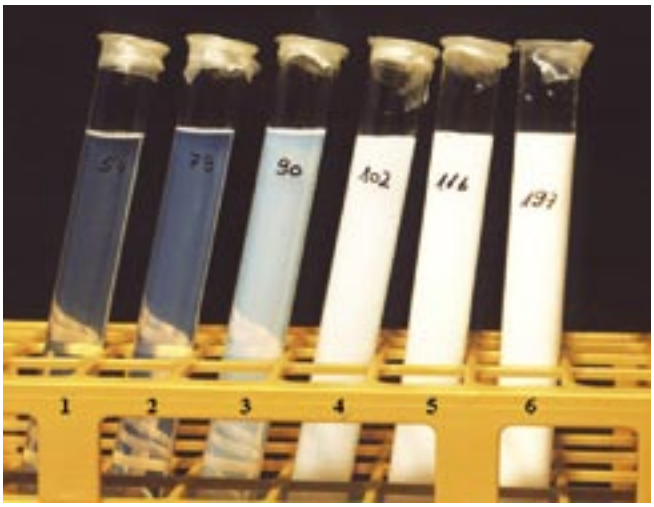

Figure 5:
Correlation between particle size and transparency. 1: 54 nm, 2: 79 nm, 3: 90 nm, 4: 102 nm, 5: 116 nm, 6: 197 nm.

Nanoemulsions containing droplets above 100 nm look white where as dispersions around 70 – 100 nm appear opaque and below that become transparent (figure 5).

Novel CoQ10 nanoemulsions

In this article we will present a novel CoQ10 double nanoemulsion with special properties manufactured according to a patented procedure. (20) The preparation consists of two different nanoemulsions. These individual nanoemulsions can now contain lipophilic compounds which are not compatible with each other such as vitamin E and Coenzyme Q10 (figure 6) which will form a dark complex when mixed together.

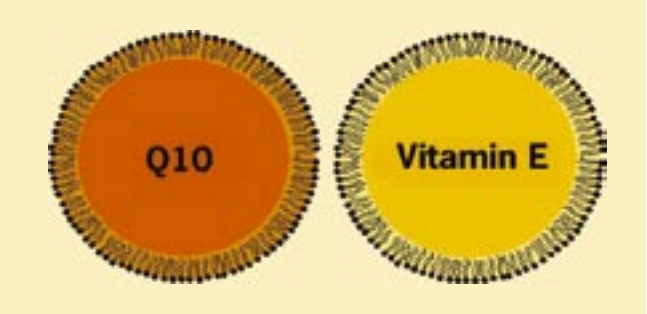

Figure 6:
Illustration of a multiple nanoemulsion containing two different inner phases. The substances of the two inner phases are not compatible with each other.

CoQ10 is obtained in its oxidized form and must therefore be reduced in the cell to act as an antioxidant. To facilitate this reaction the reducing capacity of the cell has to be enhanced by adding vitamin E (natural tocopherol). The double nanoemulsion containing CoQ10 and tocopherol in individual droplets is therefore a very smart solution to activate the cell vitality in an efficient manner. Since both individual nanoemulsions are based on a droplet size of around 50 nm the mixed preparation is transparent and has a high bioavailability.

The preparation of nanosize oil droplets offers another feature to overcome a limitation of CoQ10. The solubility of CoQ10 in oil is quite low. However, the solubility is enhanced at elevated temperature. We therefore prepared saturated CoQ10 solutions at 60 °C and used these solutions to manufacture nanoemulsions at the same temperature. Due to the small droplet size these saturated CoQ10 oil solutions remained solutions even after the temperature has been reduced to room temperature. These supersaturated CoQ10 nanoemulsions can be stored at refrigerated temperatures for many months without crystallization of CoQ10 and are therefore very interesting products for the application in cosmetics, food supplements and cell culture systems.

The use of nanoemulsions in cell culture systems offers new opportunities. Due to the small size these nanodroplets are easily absorbed by cells by means of endocytosis. This technique is described in the US patent US 6,265,180 B1 and can be applied as a novel nutritional delivery system in cell cultures. Several studies revealed that nanoemulsions containing CoQ10 in cell cultures reduce the need for blood serum, enhance the growth rate of the cells, and increase the production of antibodies. In one study the production of antibodies in hybridoma cells was enhanced by 42%. (21)

Use of CoQ10 nanoemulsions in cosmetics

CoQ10 encapsulated in the described nanoemulsions increases the synthesis of collagen I in fibroblasts. This effect was recently shown using normal human dermal fibroblasts (NHDF) and a CoQ10 nanoemulsion at a concentration of 0.1%. Cells first were cultured at standard conditions without CoQ10 during 24 hours. Then the CoQ10 nanoemulsion was added and cells were incubated for 72 hours.

The effect of CoQ10 was evaluated by visualization of the protein using a polyclonal antibody anti-collagen I and a fluorescent second antibody anti-immunglobuline-FITC. Results were photographed applying microscopy observation. The photographs show an increased secretion of collagen I compared to the control. (figure 7)

This result demonstrates that CoQ10 encapsulated in nanodroplets positively influences the expression of collagen I by fibroblasts.

In a second in vitro assay the influence of CoQ10 on the activity of mitochondrial dehydrogenase in keratinocytes was assessed. Cells (human adult low calcium high temperature cells, HaCaT) were cultured according to standard procedures and incubated for 72 hours with 0.1% of a CoQ10 nanoemulsion. In a further step cells were incubated with sodium dodecylsulfate (SDS, 2μg/ml) for 24 hours to stress the cells.

The mitochondrial dehydrogenase activity was analyzed using the MTT test. The application of the CoQ10 nanoemulsion enhanced the activity of the unstressed keratinocytes to 116.8% +/-3.2% compared to the control 100.0% +/-1.3%. The SDS treatment decreased the cell activity to 67.1% +/-4.1%.

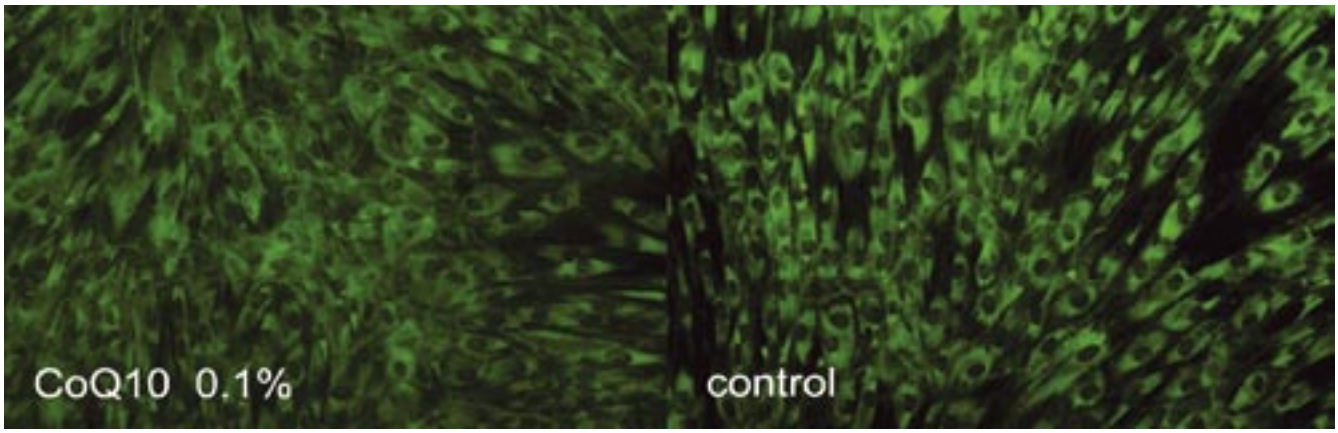

Figure 7:
Comparison of collagen I secretion by fibroblasts after incubation with CoQ10 (left) and control (right).

Pre-treatment with encapsulated CoQ10 downsized the damageing effect of SDS and the cell activity analyzed by the MTT test only decreased to 80.2% +/-1.6%. (figure 8)

The assessment reveals that CoQ10 nanoemulsions enhance the mitochondrial activity of keratinocytes and protect them against necrotic stress factors.

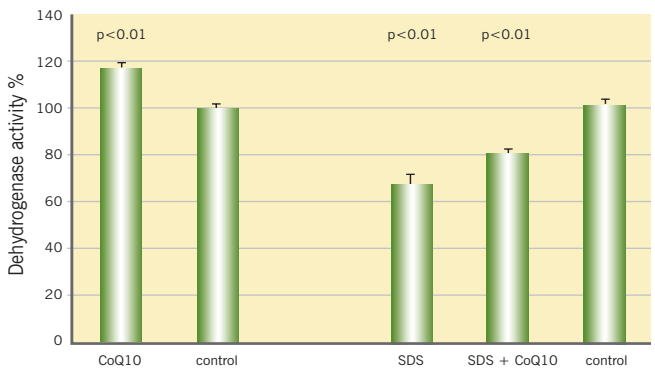

Figure 8:
Activity of dehydrogenase. Left: Influence of CoQ10 compared to control; right: influence of CoQ10 on necrotic activity of SDS.

Summary

CoQ10 proved to be a unique substance providing different possibilities of application. In medicine, CoQ10 is used for prevention and therapy of a variety of diseases. In nutrition, CoQ10 finds its advantages as a food supplement. And, as a cosmetic, CoQ10 becomes an indispensable ingredient as an antioxidant and protective agent preventing skin ageing and photo-ageing.

Since CoQ10 has a low bioavailability, strong endeavours have been made to develop efficient delivery systems. Latest research established the encapsulation of CoQ10 in nanoemulsions. Data show that the CoQ10 bioavailability is significantly enhanced by using nanoemulsions. This results in much higher CoQ10 serum levels after oral application which is of great importance for the treatment of different diseases.

The application of CoQ10 has been further improved by the development of novel CoQ10 double nanoemulsions containing tocopherol and CoQ10 in individual nanodroplets. In addition the CoQ10 concentration in these nanoemulsions could be increased by the development of a supersaturated CoQ10 nanoemulsion.

Cell Culture studies based on skin fibroblasts and keratinocytes using these novel CoQ10 nanoemulsions revealed that encapsulated CoQ10 supports the secretion of collagen I and stimulates the mitochondrial cell activity. In addition a significant protection against necrotic stress factors could also be shown.

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Primary Authors Biography

Dr. Zülli obtained Ph.D. in Biochemistry from the Swiss Federal Institute of Technology Zürich. His studies focused on structure function analysis of enzymes using methods based on molecular biology. He worked in a postdoctoral position at the Nestlé Research Center in the development of efficient expression systems of recombinant DNA in yeast.

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