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# Solubility Enhancement of Lipid-Free Coenzyme $Q_{10}$ Nanosuspension Powder

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## Introduction

Coenzyme $Q_{10}$ ($CoQ_{10}$), also known as ubiquinone or ubidecarenone, is a lipophilic substance, vitamin-like substance found in the cells of many organisms. $CoQ_{10}$ has a multifunctional role in a variety of essential cellular processes such as acting as a redox component of transmembrane electron transport systems in the respiratory chain of mitochondria, as a stabilizing agent in cellular membranes, as an essential component for production of cellular energy in the form of adenosine triphosphate (ATP) and acts as an antioxidant.[1] The cutaneous aging process resulted in the functional loss of mitochondria. $CoQ_{10}$ positively influences the age-affected cellular metabolism and opposes signs of aging. Consequently, the topical application of $CoQ_{10}$ improves mitochondria function in skin leading to benefits for human skin. However, $CoQ_{10}$ has poor water solubility because of the molecule's long side chain of 10 isoprenoid units resulted in low bioavailability. To overcome this disadvantage, many different approaches for $CoQ_{10}$ formulation have been reported as oil-based or powder-filled capsule formulations, the redispersible dry emulsion, the complexation of $CoQ_{10}$ with cyclodextrin, and self-emulsifying drug delivery systems.[1-3] Many previous studies reported the lipid component of the delivery system has great influence on its ability to enhance absorption.[3, 4] Nevertheless, most lipid-based formulations have large amounts of surfactants for enhanced drug absorption.[3, 5, 6] Lipid-free $CoQ_{10}$ nanosuspensions stabilized by various surfactants, i.e., soybean lecithin (SL), D-$\alpha$-Tocopherol polyethylene glycol 1000 succinate (TPGS), Polyoxyl 40 hydrogenated castor oil (PHCO), Polyglycerol 10 stearic acid ester (PSAE), sucrose palmitate (SP), achieved similar or higher levels of $CoQ_{10}$ bioavailability than that reported for a lipid-based nanoemulsion.[3] Lipid-free $CoQ_{10}$ nanosuspensions stabilized by TPGS showed the smallest particle size with good stability for 6 months.[7]

Therefore, the aim of this study was to enhance the solubility of $CoQ_{10}$ powder by development of lipid-free $CoQ_{10}$ nanosuspensions stabilized by TPGS. Lipid-free $CoQ_{10}$ nanosuspension was transferred into dry powder by lyophilization. To prove the solubility enhancement of $CoQ_{10}$, the determination of $CoQ_{10}$concentration was analyzed by high performance liquid chromatography (HPLC), and the physicochemical properties characteristic of lipid-free $CoQ_{10}$ nanosuspensions and dry powder were evaluated by proton correlation spectroscopy (PCS), transmission electron microscopy (TEM), differential scanning calorimetry (DSC), and X-ray diffraction (XRD) to supporting data of $CoQ_{10}$ solubility enhancement.

## Methods

### *Production of lipid-free $CoQ_{10}$ nanosuspension*

As bottom up process, 2 % w/w $CoQ_{10}$ was dissolved in 47 % w/w of 96 % ethanol in glass syringe, and dropped into the chamber of Microfluidizer (Microfluidics, Newton, USA) that was contained 1 % w/w TPGS solution at a flow rate of 1 ml/min for 50 minute. As top down process, the lipid-free $CoQ_{10}$ nanosuspension was produced by microfluidization method at 55 ± 2 °C, applying pressure at 600 bar (equal to 6,000 kPa and 8,702 psi).

### *Particle size and Zeta-potential analysis*

Particle size and zeta-potential analysis of lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS were performed with a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) by a dynamic light scattering method. Before the measurement, samples were diluted with distilled water to have a suitable scattering intensity. Mean diameter and size distribution was calculated using the photon correlation from light scattering. All measurements were performed at 25 °C at an angle of 173°. PCS yields the mean particle size (z-average)

and the polydispersity index (PdI), which is a measure of the width of the size distribution. Applying a combination of Laser Doppler Anemometry and Phase Analysis Light Scattering was used for zeta-potential measurement. The samples were diluted with distilled water adjusted with 0.9% (w/v) sodium chloride solution to a conductivity of 50 µS $cm^{-1}$. The pH was in the range of 5.5 to 6.0. The mean value of the zeta potential and the standard deviation of 10 measurements are given.

***Lyophilization of lipid-free $CoQ_{10}$ nanosuspension***

The aqueous lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS was dried by lyophilization. Briefly, 1 ml of the aqueous lipid-free $CoQ_{10}$ nanosuspension in a 20 ml vial was frozen at −70 °C. The frozen lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS was dried out by lyophilyzer using a Christ® Alpha I-5 (Martin Christ Gefriertrocknungsanlagen GmbH, Germany) for 24 hrs, without applying a secondary drying process.

***Transmission electron microscopy***

The morphology of lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS was assessed by using a TEM using TECNAI G2 20 S-TWIN (FEI, USA). The samples were prepared by placing a drop of lipid-free $CoQ_{10}$ nanosuspensions stabilized by TPGS onto a 400-mesh copper grid coated with carbon film, followed by negative staining with 1.5 % phosphotungstic acid. Then, the samples were dried in air without vacuum before TEM analysis.

***X-ray diffraction (XRD)***

X-ray diffractometer (wide angle scattering-WAXD, Philips, Amedo, the Netherlands) was used for diffraction studies of substances. XRD studies were performed on the samples by exposing them to a copper anode (Cu-Kα radiation, 40 kV, 25 mA, $\lambda = 0.15418$ nm) and scanned from 0.6° to 40° at 2θ. Prior to measurement, 1.0 ml of lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS was admixed with 0.2 g of locust bean gum powder to produce a paste, and then mounted into a glass fiber. The data used was typically collected with a step width of 0.04° and a count time of 60 s.

***Differential Scanning Calorimetry (DSC)***

To evaluate polymorphism of lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS, DSC analysis was performed by using a Mettler DSC 821e apparatus (Mettler Toledo, Germany). The samples weighing approximately 1 - 3 mg based on the solid content were placed in 40 µl aluminium pan. An empty aluminium pan was used as a reference. The heating runs were performed from 20 to 80 °C and cooled to 0 °C using the heating rate of 10 K/min by flushing with nitrogen at the rate of 80 ml/min.

***Solubility studies***

Saturated solutions of the raw $CoQ_{10}$ and the powder of $CoQ_{10}$ nanosuspension stabilized by TPGS $CoQ_{10}$ were prepared by adding excess both samples into water and using a shaker (InnovaTM 4230, New Brunswick Scientific Edision, NJ-USA) 100 rpm at 25 °C for 48 h. Both samples were then centrifuged by microcentrifuge (Biofuge 22 R, Heraeus Surgical, USA) with 17000 rpm at 25°C for 30 minute, and the supernatants were collected, appropriately diluted and determined for $CoQ_{10}$.Then, the solubility of both samples in water were analyzed by HPLC at the wavelength of 280 nm on a Kroma System 2000 (Kontron Instruments, Berlin, Germany) running in the isocratic mode. Kontron HPLC software was used to perform integration of the peaks. The system consisted of a Betasil® C8 RP column (Thermo electron corporation, Germany). Column temperature was maintained at 20 °C. The standard and samples were separated using an isocratic mobile phase consisting of 90 parts of acetonitrile and 10 parts of tetrahydrofuran. The injection volume was 20 µl, the flow rate 1.5 ml/min. The running time was 10 minutes.

## Results

***Production of lipid-free $CoQ_{10}$ nanosuspension***

Lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS was produced by the combination of bottom up and top down approaches using microfluidization method. In the bottom up approach, $CoQ_{10}$ was dissolved in 96 % ethanol then dropped to TPGS solution that caused the precipitation of $CoQ_{10}$. In the subsequent top down approach, the particle size of the precipitation of $CoQ_{10}$ was decreased by microfluidization method. As a result, the macroscopic appearance of lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS is translucent yellow as shown in Figure 1.

**Figure 1** Macroscopic appearance of lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS

***Particle size and Zeta-potential analysis***

Figure 2 shows the particle size and polydispersity index of lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS were 69.8 ± 1.9 nm and 0.17 ± 0.03. The zeta-potential was -24.9 ± 1.2 mV. The particle size, polydispersity index, and zeta-potential of the redispersed powder of lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS were 169.7 ± 13.0 nm, 0.15 ± 0.04, and -1.2 ± 0.8 mV, respectively.

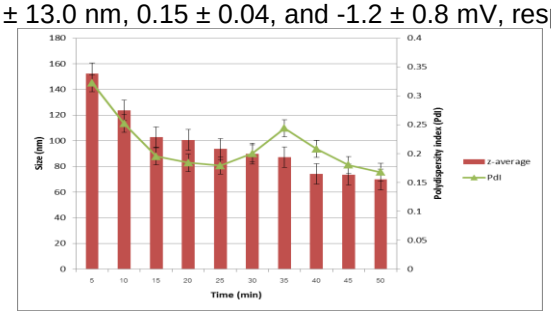


**Figure 2** Particle size and polydispersity index of lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS

***Lyophilization of lipid-free $CoQ_{10}$ nanosuspension***

Lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS was transferred to dry powder by lyophilization. It was found that 20 mg of the powder of lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS was obtained from 1 ml of lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS. The powder of lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS is light yellow (B) compared to the raw $CoQ_{10}$ that is orange (A) as shown in Figure 3.

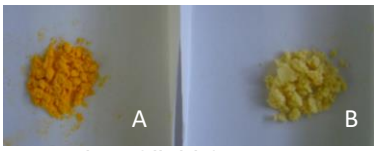


**Figure 3** A - the raw $CoQ_{10}$ and B - powder of lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS

***Transmission electron microscopy***

Figure 4 shows the morphology of the raw $CoQ_{10}$, lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS, and the redispersed powder of lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS. The rectangular prism of the raw $CoQ_{10}$ was observed by light microscopy at 100x magnification. TEM examinations clearly indicate the spherical shape of lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS and the redispersed powder of lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS. From TEM results, the aggregation of the redispersed powder of lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS was shown.

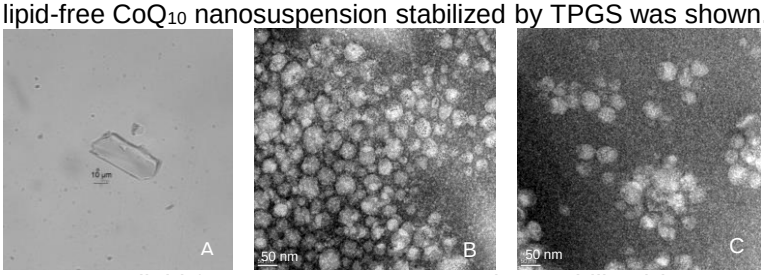


**Figure 4** A - the raw $CoQ_{10}$, B - lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS, and C - redispersed powder of lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS

***X-ray diffraction (XRD)***

According to the XRD patterns in Figure 5, sharp and intense peaks were observed in x-ray diffractogram at ca 18° (2Θ) for the raw $CoQ_{10}$ that should be identified the crystalline condition of the raw $CoQ_{10}$. On the other hand, a significant reduction in disparity of highs and lows was shown in x-ray diffractogram. This suggests that a lack of crystallinity in the powder of lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS or lyophilized $CoQ_{10}$.

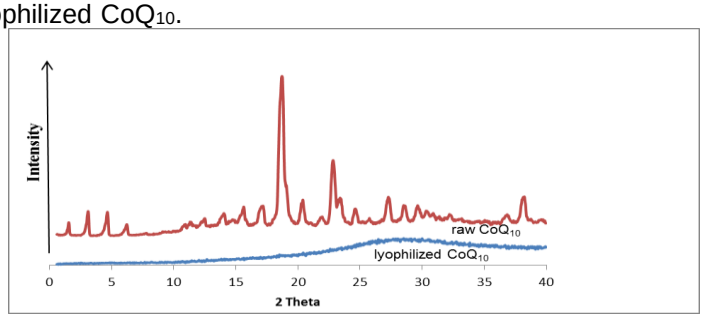


**Figure 5** X-ray diffractogram of the raw $CoQ_{10}$ and lyophilized $CoQ_{10}$

***Differential Scanning Calorimetry (DSC)***

DSC thermogram of the raw $CoQ_{10}$ and TPGS shows an intense endothermal peak at ca 50 °C and 41°C; however, the intensity of the endothermal peak of the lyophilized $CoQ_{10}$ is lower than the raw $CoQ_{10}$ and TPGS as shown in Figure 6.

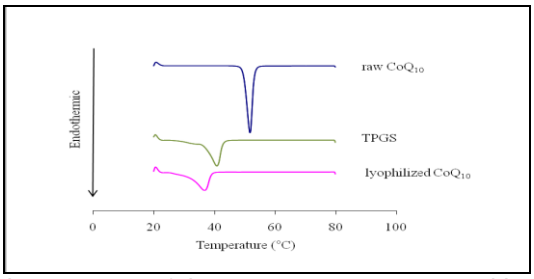


**Figure 6** DSC thermogram of the raw $CoQ_{10}$, TPGS, and lyophilized $CoQ_{10}$

***Solubility studies***

The saturation solubility of the powder of lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS and the raw $CoQ_{10}$ was analyzed by HPLC. The solubility of the powder of lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS was 3.32 ± 0.08 mg/ml, and retention time was 7.52 ± 0.02 min, whereas the raw $CoQ_{10}$ in water was not detected due to the very low solubility of the raw $CoQ_{10}$ in water. The solubility of the raw $CoQ_{10}$ should be lower than 0.035 µg/ml that is the limit of detection (LOD) of this HPLC.

## Discussion

In this study, lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS was produced by the combination of bottom up and top down approaches using microfluidization method. The particle size and polydispersity index of lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS was less than 100 nm with a narrow dispersity index. The negative charge was the effect of the hydrophilic group of TPGS that preferentially adsorbs hydroxyl ion on the surface of particles.[8] The decrease in zeta-potential (< - 20 mV) induced the aggregation of the redispersed powder of lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS.[5] However, the solubility of the powder of lipid-free $CoQ_{10}$ nanosuspension stabilized by TPGS was about 100,000 times greater than the raw $CoQ_{10}$.It could be explained by both physical treatments, i.e. bottom up and top down approaches, and stabilizer, i.e. TPGS, affected the particle size and the crystalline state of $CoQ_{10}$ resulted in solubility enhancement of $CoQ_{10}$.[9]

## Conclusion

This study demonstrated that the solubility of $CoQ_{10}$ was enhanced by lipid-free nanosuspension stabilized by TPGS. This carrier should be further developed for an alternative cosmeceutical product in the future.

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