Preparation and Characterization of Coenzyme Q_{10}-Loaded Nanostructured Lipid Carriers as Delivery Systems for Cosmetic Component

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ABSTRACT

Coenzyme Q_{10} (CoQ_{10}) is practically insoluble and chemically labile in water and should be incorporated in some carriers such as NLC to improve its stability and realize the final application as a versatile anti-oxidant. CoQ_{10}-NLC (Nanostructured Lipid Carrier) was prepared based on the phase behavior of a special hot transparent emulsion containing CoQ_{10}. The good physical stability of particle was obtained by Photon Correlation Spectroscopy (PCS). UV measurements sho-wed that the loading capacity of CoQ_{10} could reach 5%. The 99.8% entrapment efficiency (UV analysis) was obtained for all formulations. Atomic Force Microscope studies revealed NLC of pellet-like shape.

Keywords: coenzyme Q_{10}, nanostructured lipid carriers, phase diagram, entrapment efficiency

1. INTRODUCTION

Nanostructured Lipid Carrier (NLC) is a new improved lipid carrier following Solid Lipid Nanoparticles (SLN) [1-4]. Based on the chemical nature of lipid molecules, the inner structure of NLC is different from that of SLN because the former is composed of mixtures of solid and liquid or solid and solid lipids [5]. The proportion of oil phase in NLC is generally much higher than in solid lipids. Hence the higher loading capacity could be achieved by NLC. NLC could keep solid skeleton at body temperature by changing the proportion between solid and liquid lipids [2], therefore drug release can be controlled [7]. With this approach, the minimization of drug expulsion is also achieved during storage time.

CoQ_{10} is a quinine compound that is widely biosynthesized in living organisms such as yeasts, plants, and animals [8], and it is lipophilic [9]. CoQ_{10} can improve cellular dynamics in human body and play an effective role in preventing skin aging, keratinization and DNA oxidative damage induced indirectly by UVA [9, 10]. Furthermore, CoQ_{10} has functions of protecting and providing alimentation to skin. Reactive Oxygen Species (ROS) produced in human body can be restrained and the peroxidation of lipid could also be slowed and even prevented by CoQ_{10} [9], and its effect is better than that of V_E and V_{B2}. CoQ_{10} become a hot topic in cosmetics because it can accelerate cell division and rebirth, and stimulate cell activity, so relive cell of skin would be possible.

2. MATERIALS AND METHODS

2.1 Materials

CoQ_{10} was purchased from Haotian Bioengineering Technology Co., Ltd. (Xi'an, China), O/100G (Trade Name) and O/020G (Trade Name) were purchased from Xianglin Enterprise Co.,Ltd.(Guangzhou, China), Glycerin mono-stearate, Glyceride, ethanol and span 20 were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Other reagents were all commercial available products. MilliQ water was used in all the experiments.

2.2 Preparation of CoQ_{10}-NLC

CoQ_{10}-NLC containing 5% (w/w) CoQ_{10} was produced by hot high pressure homogenization (HPH). Briefly, after melting the lipid phase at a temperature of 65 °C, 5% of CoQ_{10} (% with regard to the emulsion) was added until thoroughly dissolved and this mixture was immediately dispersed in a hot surfactant solution using an Ultra-Turrax FM200 (FLUKO, Germany) at 8000 rpm for 20 s. The pre-emulsion was further processed by high pressure homogenizer (ATS 100D, Canada) using seven homogenization cycles at 600 bar and 65 °C. The obtained nanoemulsions were cooled to room temperature to crystallize the lipid and finally formed the active-loaded NLC.

2.3 Particle size analysis

Particle size analysis was conducted within 24 h after preparation (day 0) and the physical stability was monitored during a period of 3 months storaged at 4 °C, room temperature (25 °C) and 40 °C. Photon correlation spectroscopy (PCS) was performed using Malvern Zetasizer ZS90 (Malvern Instruments, UK) and laser diffractometry (LD) was achieved by the Mastersizer 2000 (Malvern Instruments, UK). PCS mean diameter, polydispersity index (PI) and D10, D50 and D90 diameters were used as qualitative parameters of the obtained aqueous dispersions. The LD values represent the percentage of the particles that is smaller than the given size.

2.4 Loading capacity

The UV spectrum of CoQ_{10} was obtained by wavelength scanning from 250 to 750 nm using a UV-2450 double-beam spectrophotometer from Shimadzu Corporation (Kyoto, Japan). CoQ_{10}-NLC was analyzed after dissolution in ethanol

2.5 Entrapment Efficiency

The entrapment efficiency (E.E.) of CoQ_{10}-NLC was measured by ultrafiltration with centrifugal filter tubes with a molecular weight cut-off of 30 kDa (Millipore, America). The E.E. was calculated by the difference between the total amount of CoQ_{10} used to prepare the systems and the amount dissociating in the systems, applying Eq.:

$$E.E. = \frac{Total\ amount\ of\ CoQ10 - Free\ amount\ of\ CoQ10}{Total amount of\ CoQ10} \quad (1)$$

The amount of CoQ_{10} was determined by UV analysis.

2.6 Atomic Force Microscope

The shape of CoQ_{10}-NLC was observed by Atomic Force Microscope (AFM, VEECO Dimension 3100 Atomic Force Microscope, American). Prior to analysis, samples were diluted by bidistilled water until the color of CoQ_{10}-NLC disappeared, and then the diluted samples were dropped on isinglass and dried under a nitrogen stream.

3. RESULTS AND DISCUSSION

3.1 Preparation of CoQ_{10}-NLC

O/100G and O/020G were selected as emulsifiers. The proportion of the two emulsifiers was obtained by the phase diagram of the O/100G/O/020G/GMS/Glyceride/CoQ_{10} system (Fig. 1).

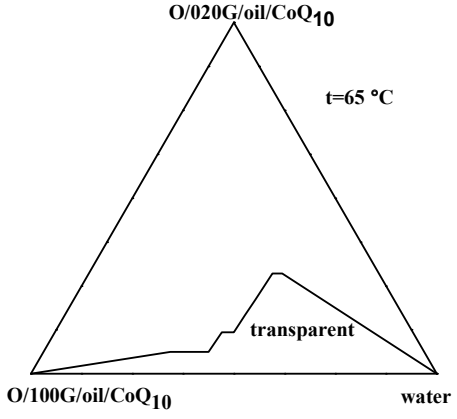

Figure 1: Pseudoternary phase diagram of O/100G/O/020G/GMS/Glyceride/CoQ_{10} system

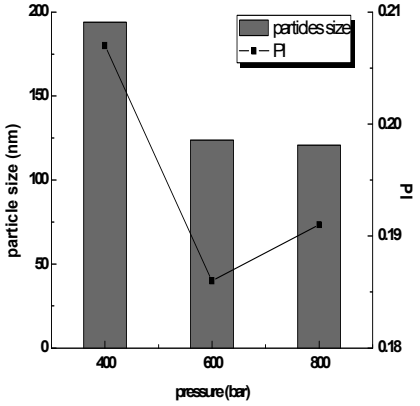

Figure 2: Relation of mean particle size of CoQ_{10}-NLC, HPH pressure and particle size distribution.

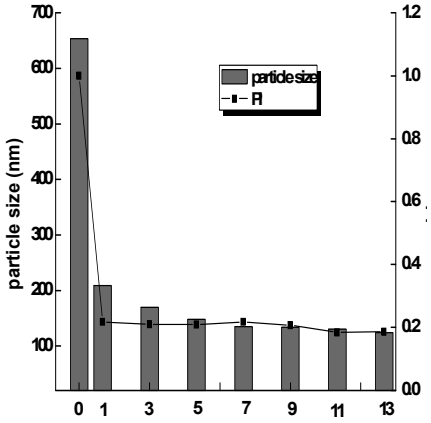

Figure 3: Relation of mean particle size of CoQ_{10}-NLC, homogenization cycle of HPH and particle size distribution.

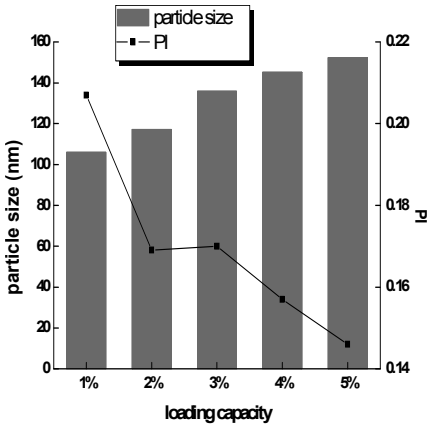

Figure 4: Relation of mean particle size of CoQ_{10}-NLC, loading capacity of O/100G/O/020G/GMS/Glyce-

ride/CoQ_{10} and particle size distribution

The temperature was kept at 65 °C because melting point of oil phase in this system is near 55 °C. The result of HPH investigation indicated that the particle size of CoQ_{10}-NLC was negatively correlated with the pressure (Fig. 2). The relation between HPH cycle and particle size of CoQ_{10}-NLC is shown in Fig. 3. Apparently, the particle size minished accompanied by the increase of homogenization cycle. The particle size can be less than 160 nm when the system was homogenized 7 cycles, and it almost didn't change even if the system was homogenized more than 7 cycles. So we chose 600 bar and 7 cycles as the preparation condition. Fig. 4 showed that the amount of loaded CoQ_{10} increased with the particle size increasing when proportion between the oil and emulsifiers was fixed, and all of the zeta positions are near to – 6.00 mV.

3.2 Particle size stability and zeta potential

NLCs with 5% (w/w) CoQ_{10} were prepared at different temperatures. As shown in Table 1, the obtained volume distribution diameter of 90% CoQ_{10}-NLCs particles which were respectively prepared at 55, 65 and 75 °C was below 280 nm. And the difference in particle size of the three types of CoQ_{10}-NLC was very small. So the preparation temperature doesn't have a great impact on particle size. To investigate the influence of storage temperature on the particle size stability, the CoQ_{10}-NLCs were stored at 4, 25 and 40 °C, respectively. Table 2 shows the size parameters of the formulation prepared at 65 °C during 3 months of storage at 4, 25 and 40 °C, respectively. Apparently, CoQ_{10}-NLCs' particle sizes were stable during 3 months at any storage temperature. And PI values were less than 0.250 for all formulations, indicating a relatively narrow size distribution.

Formulations	D10 (μm)	D50 (μm)	D90 (μm)
CoQ10-NLC ($P_{55\ °C}$)	0.142	0.194	0.276
CoQ10-NLC ($P_{65\ °C}$)	0.139	0.188	0.263
CoQ10-NLC ($P_{75\ °C}$)	0.138	0.186	0.259

Table 1: Volume distribution diameters in micrometer (μm) (D10, D50, D90) obtained by LD (at 25 °C) during the day of production.

Parameters	Storage time	$P_{65\ °C}$/ $S_{4\ °C}$	$P_{65\ °C}$/ $S_{25\ °C}$	$P_{65\ °C}$/ $S_{40\ °C}$
PCS (nm)	Day 0	152	159	149
	Day 1	159	159	152
	Day 15	160	162	159
	Month 1	161	162	160
	Month 2	167	165	160
	Month 3	169	170	175
PI	Day 0	0.226	0.159	0.176
	Day 1	0.181	0.181	0.209
	Day 15	0.168	0.171	0.181
	Month 1	0.209	0.220	0.195
	Month 2	0.161	0.176	0.171
	Month 3	0.169	0.160	0.172

Table 2: PCS data of CoQ_{10}-NLC prepared O/100G/O/020G/GMS/Glyceride/CoQ_{10} system (P means production temperature and S means storage temperature).

Formulation	C time(min)				particle size (nm)	
	5	10	20	30	before C	after C
d 0-fold	-	-	-	-	145	146
d 5-fold	-	-	-	-	146	146
d 20-fold	-	-	-	-	144	147

Table 3: Phenomena of aqueous CoQ10-NLC dispersions prepared as O/100G/O/020G/GMS/Glyceride/CoQ10 system after high-speed centrifugation (10000 rpm) and changes of particle size of CoQ10-NLC before and after centrifugation ("-" means no new phenomena, "d" means to diluted, "C" means to centrifugation)

From Table 3, we can discover that the formulation have no change after high-speed centrifugation for 30 min at 10000 rpm. The particle size of CoQ10-NLC was similar before and after centrifugation. It was the same to the aqueous CoQ10-NLC dispersions that were diluted 2, 5, 10 and 20-fold. So the Physical properties of aqueous CoQ10-NLC dispersions and their diluent prepared as O/100G/O/020G/GMS/Glyceride/CoQ10 system are very stable.

3.3 Loading capacity and entrapment efficiency

CoQ_{10}-loaded NLC dispersions were scanned at the wavelength of 200 to 500 nm. Since NLC components do not absorb UV radiation when dissolved, the typical absorption peak for CoQ_{10} is clearly visible at 275 nm in the tested formulation.

An appropriate calibration curve of cosmetic active in ethanol in concentrations of 0.1–0.8 mg/mL was obtained for UV absorption spectroscopic analysis (Fig. 7). According to this calibration curve, the loading capacity of CoQ_{10}-NLC of 52.3 mg/mL was obtained.

An amount of ~ 0.099 mg/mL free CoQ_{10} in aqueous CoQ_{10}-NLC dispersion was obtained. Thus, it can be deduced that 99.8% E.E. was assumed in all formulations.

The high E.E. was the result of high lipophilicity of Q_{10} and its low solubility in water.

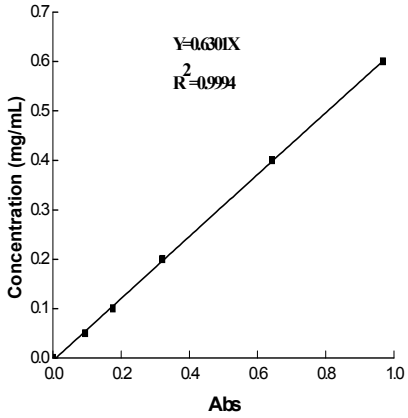

Figure 7: Calibration curve of CoQ10 in ethanol in concentrations of 0.05–0.8 mg/mL

3.5 Atomic Force Microscope

In order to study the particle size and shape distinctly, AFM (VEECO Dimension 3100 Atomic Force Microscope, American) analysis was used. Fig. 8 shows the image of Q_{10}-loaded NLC. Particles display a regular pellet.

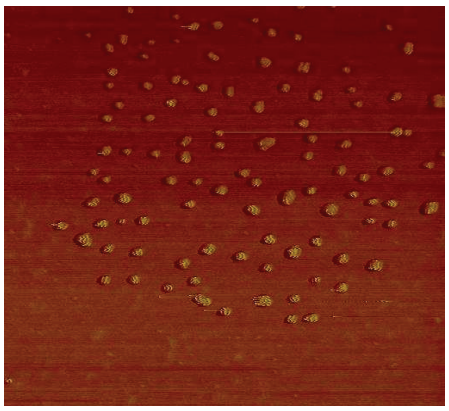

Figure 8: AFM image of CoQ_{10}-NLCs

4. CONCLUSIONS

CoQ_{10} is one of the most unstable actives for anti-ageing. Because of the high lipophilicity and its low solubility in water, it is possible for incorporate CoQ_{10} into lipid nanoparticles. Based on the study of different phase systems, O/100G, O/020G and Span20 were chosen as emulsifiers, and GMS, and Glyceride were selected as the lipids. Particle sizes had no obvious change with storage of the aqueous dispersions at different temperatures for 3 months. And the particle sizes also were nearly invariable after some excessive methods such as high-speed centrifugation and dilution by buffer solutions with different pH. The results of particle size analysis indicated that physical characteristics of aqueous CoQ_{10}-NLC dispersions were stable. In the process of preparation, 4.78% (w/w) CoQ_{10} was contained in a topical formulation and this mass ratio was a sufficiently high concentration to the aqueous CoQ_{10}-NLC dispersions. These regular pellet-shape nanoparticles had good envelopment effect, with an entrapment efficiency of 99.8% by UV analysis.

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