Research Article

Kolliphor® HS 15 Micelles for the Delivery of Coenzyme Q10: Preparation, Characterization, and Stability

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ABSTRACT. To enhance the stability of coenzyme Q10 (CoQ₁₀), Kolliphor® HS 15 (HS15) was employed as a carrier to build up a stable CoQ₁₀-loaded micelle delivery system. The impact of micellar compositions, the preparation condition, and the preparation method on size characteristics, the solubilization efficiency, and micellar stability were investigated. The optimal preparation conditions were 1:6, 4, 0.2%, 118°C, and 25 min for CoQ₁₀/HS15 mass ratio, pH value, the concentration of glucose, and the sterilization conditions. Upon these conditions, the particle size, polydispersity index (PDI), zeta potential, the entrapment efficiency, drug loading, and the critical micelle concentration (CMC) of CoQ₁₀-loaded micelles were 19.76 nm, 0.112, −3.405 mV, 99.39%, 13.77%, and 5.623×10^{-4} g/mL, respectively. Differential scanning calorimetry (DSC) analysis collectively corroborated that CoQ₁₀ was entrapped into the micelles in amorphous form. The release pattern of drug was analyzed and proved to follow the first order. Additionally, the samples were exposed to the temperatures of 30°C for 6 months with more significant impact on their stabilities as compared to 4 and 25°C based on particle size and PDI. Under constant humidity with light protection long-term (25±2°C, relative humidity (RH) 60±10%, 18 months) conditions, there was no variation except minor changes of CoQ₁₀ content of the samples. The shelf life of the micellar samples could be predicted as 24 months based on the stability results. Consequently, the CoQ₁₀-loaded micelles showed excellent stabilities below 25°C as a potential drug candidate for further clinical applications.

KEY WORDS: coenzyme Q₁₀; Kolliphor® HS 15; micelles; preparation; stability.

INTRODUCTION

Coenzyme Q10 (CoQ₁₀) is a fat-soluble vitamin analog found in the cells of many organisms, especially the inner mitochondrial membrane (1). In eukaryote, it functions as a cofactor in the electron transport chain and serves as a modulator of the transition pore (2). Hence, CoQ₁₀ is a well-known antioxidant, which regenerates alpha-tocopherol, another critical antioxidant, scavenges free radicals, and inhibits lipid peroxidation (3). Nevertheless, the CoQ₁₀ level is often found to be deficient in several disease forms (4). CoQ₁₀ is now diffusely applied as a supplement in cardiovascular health (5).

In spite of its versatility, poor bioavailability is one of the limiting factors in the clinical development of CoQ₁₀ (6) which is mainly caused by its extremely low solubility in water (<0.25 µg/ml) (7,8). Various efforts have been made for improving the solubility of CoQ₁₀ using a capable vehicle to enclose hydrophobic, such as inclusion complexes with microemulsions, dendrimers, or protein formulation (7,9–11). The administration of many cosolvents or surfactants causes toxicity and other undesirable side effects (12). Another option is to use certain micelle-forming surfactants in formulations of insoluble drugs. Thus, the requirement of an ideal drug delivery system comprises efficient and stable solubilization, which enhances the solubility of hydrophobic solutes via micellar formation at the concentrations above the critical micelle concentration (CMC). As a drug vector, micellar carrier is one of the most important entities designed essentially for effective transportation of loaded drug to the target site, which sequesters transport and retains the active drug until its site-specific delivery (13). Polymeric micelles by amphiphilic diblock copolymers are generally more stable and safer than conventional surfactant ones. However, critical problems relating to toxicity, caused by residual monomers or oligomers, and the drug-to-monomer cross-reaction may limit potential use (11,14). Besides, the complexity of preparing limits the development of clinical studies. The preparation of conventional surfactant micelles is simple and feasible, on

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the contrary. Hence, exploring an efficient and stable solubilizer is still desired.

Solubilizers currently used for parenteral administration such as Cremophor® EL and polysorbate 80 have been implicated in clinically important adverse effects such as hypersensitivity reactions and a highly increased systemic drug exposure along with a reduced cellular uptake (15). To overcome these problems, they are replaced with a new nonionic surfactant called Kollipher® HS 15 (HS15; polyglycol mono-ester and diester of 12-hydroxystearic acid with about 30% free polyethylene glycol) (16), which is known to be safe in various animal toxicity models with reduced histamine levels compared with Cremophor® EL and polysorbate 80 (17). HS15 is official in the German Pharmacopoeia (DAB), US Pharmacopoeia (USP), and European Pharmacopoeia (Ph. Eur) monographs, and it has an import registration certificate in China. HS15 has been found to enable poorly soluble drugs to enhance the solubilization (18–20) and to inhibit to some degree the P-glycoprotein efflux mechanism (21,22). Thus, HS15 greatly increases the transportation of drug in the systemic circulation for poorly soluble drugs, further boosting the bioavailability.

In this contribution, HS15 was applied to produce a novel CoQ10-loaded micellar system to enhance the physicochemical stability of CoQ10. CoQ10, a promising antioxidant, was controllably incorporated within the micelles, since the marketed formulation Hefu® was easy to crystallize. The physical and chemical properties including major parameters as the particle size, the particle dispersion index (PDI), zeta potential, the content of CoQ10, and the related substance have been investigated to optimize the preparation conditions of CoQ10-loaded micellar system. The obtained CoQ10-loaded micelles were evaluated by size characteristics, solubilization efficiency, and the micellar stability. Finally, the shelf life of 24 months was obtained, and the CoQ10-loaded micelle system has good stability under 25°C.

MATERIALS

Materials

CoQ10 was purchased from Shanxi Sciphar Hi-Tech Industrial Co., Ltd. (Shanxi, China); HS15 was kindly supplied by BASF Co., Ltd. (Shanghai, China). Polysorbate 80 was obtained from Nanjing Well Chemical Co., Ltd. (Nanjing, China). Methanol and ethanol were purchased from Spectrum Chemical Mfg. Corp. (HPLC grade, Gardena, USP).

Methods

Solubilization Capacity Determination

Excess amounts of CoQ10 were respectively added to 10 mL of different micellar solutions (HS15 or polysorbate 80) in vials which were then shaken in a thermostatted water bath (DF-101S; Kefeng Equipment Co., Ltd., Zhengzhou, China) at 50°C for 24 h. Then, excess amounts of the poorly soluble CoQ10 were separated by 10 min centrifugation at 10,000 rpm in a centrifuge (H2050R-1; Xiangyi Centrifuge Machines Ltd., Hunan, China). CoQ10 concentration in the supernatant solutions was determined by using HPLC (23).

All data were performed as mean ± standard deviation (SD), $n=3$.

Determination of Hemolytic Activity of HS15

The hemolytic activity of HS15 micelles was investigated by rabbit red blood cells (RBCs) with polysorbate 80 used as a control. Briefly, RBC suspension (25 mL) was added into 25 mL of samples to make the concentrations of HS15 and polysorbate 80 of 120, 240, and 600 µg/mL. The samples were incubated at 37°C for 24 h, and 6 mL of the solution was taken at a desired time interval and then centrifuged at 3000 rpm for 10 min to remove intact RBCs. The supernatant was analyzed for released hemoglobin with a UV-1800 spectrophotometer (Mapada, China) at 540 nm. Normal saline and distilled water were added to obtain 0 and 100% hemolysis, respectively. The degree of hemolysis was calculated by the following equation:

$$\text{Hemolysis}(\%) = \frac{A_s - A_0}{A_{100} - A_0} \times 100$$

Specifically, A_s is the absorbance of a sample, and A_{100} and A_0 are the absorbance of the reference solutions at 100% hemolysis and 0% hemolysis, respectively (24).

Preparation of CoQ10-Loaded Micelles

The preparation method consisted of the following steps. In brief, specified amounts of HS15 (from 10 to 40 mg) and CoQ10 (5 mg) in a glass beaker were dissolved in a thermostatted water bath at 50°C and stirred for 15 min. Then, 3 mL of 0.1538 M sodium chloride solution containing stabilizer was added drop by drop while stirring. Subsequent solution was introduced for diluting the micellar solution to 50 mL. Thus, the pH of the solution was adjusted with 0.1 M HCl and NaOH. The samples were filtered using a syringe filter. Eventually, the subjects were sterilized as shown in subsequent section.

In the preliminary experiments, such influence factors such as types and amount of solubilizer, temperature, hydration time, and volume were investigated to identify the control factors and their levels during assembly process with main particle size, PDI, entrapment efficiency (EE%), and so on as index. According to the influence of each factor, the ratio (w/w) of CoQ10 and HS15, the amount of stabilizer, pH value, and the condition of sterilization served as four key factors to obtain the optimized formulation.

Screening of HS15 Concentration. CoQ10 was dissolved in HS15, in ratios of 1:2, 1:3, 1:4, 1:5, 1:6, 1:7, and 1:8, respectively. The most suitable ratio was identified on the basis of transmittance, the particle size, PDI, entrapment efficiency, and drug loading capacity.

Screening of Suitable Stabilizer and Its Concentration. CoQ10-loaded micelles were prepared by using different types and concentrations of stabilizers such as glucose, cysteine, thiourea, and sodium pyrosulfite. The ratio of CoQ10 to HS15 (1:6) was kept constant. The most suitable stabilizer and its concentration were

identified based on the content of CoQ10 and the related substance.

Screening of pH. Screening of pH aimed to get the optimum CoQ10-loaded micelles. Toward this purpose, micelles were prepared following the above described process with the ratio of CoQ10 to HS15 at 1:6 and the glucose concentration at 0.2% (w/v). Different pH values were adjusted for preparation of micelles, and optimum pH values were selected based on the particle size, PDI, zeta potential, content of CoQ10, and related substance.

Optimization of Sterilization. CoQ10-loaded micelles were prepared using different sterilization conditions, i.e., 25 min at 118°C and 20 min at 121°C, and its effect on the particle size, PDI, zeta potential, content of CoQ10, and related substance were studied. Other experimental parameters, including micellar compositions (CoQ10/HS15 (w/w) 1:6), glucose concentration (0.2% w/v), and pH 4.0, were kept constant.

Characterization of CoQ10-Loaded Micelles

Particles Size and Zeta Potential Measurement. The micellar sizes, size distributions, and zeta potential were determined by dynamic light scattering (DLS) using a Zetasizer Nano (Malvern, UK). To be specific, samples were analyzed at 25°C at an angle of 90°.

Entrapment Efficiency and Drug Loading Capacity. Micellar entrapment efficiency (EE%) and drug loading capacity (DL%) were determined using the centrifugation technique (25) for separating the nonentrapped drug from micelles. The content of CoQ10 in CoQ10-loaded micelles (without centrifugation was m_1 , with centrifugation was m_2) was determined by using a validated HPLC method (1260, Agilent Technologies Inc., USA). The content of unloaded micelles is m_3 . Micelles were achieved using a reversed phase C18 ODS-SP column (15 cm×4.6 mm, Inertsil, JAP). The mixture of ethanol/methanol (1:1 v/v) was used as the mobile phase at a flow rate of 1 mL/min with UV detection at 275 nm:

$$EE\% = \frac{m_2}{m_1} \times 100\%; \quad DL\% = \frac{m_2}{m_1 + m_3} \times 100\%$$

DSC Analysis. The differential scanning calorimetry (DSC) thermogram of the pure CoQ10, HS15, CoQ10-loaded micelles, and physical mixture of CoQ10 and HS15 was performed using a NETZSCH apparatus (DSC200PC, NETZSCH, Germany). Measurements were conducted at a heating rate of 10°C/min from 25 to 350°C. The thermal behavior was studied by heating about 3 mg of samples in an aluminum pan under nitrogen gas. An empty aluminum pan was utilized as a reference (26).

Determination of CMC. CMC was determined by the pyrene fluorescence method with slight modification from the reported method (27). From a stock solution of 5×10^{-4} mol/L pyrene dissolved in acetone, 0.1 mL aliquots were

transferred with a micropipette into a series of clean, dry test volumetric flasks, and the solvent was allowed to evaporate under nitrogen. A series of unloaded and CoQ10-loaded micellar solutions were added to dry pyrene. Pyrene concentration in each volumetric flask was 5×10^{-6} mol/L. The mixtures were shaken in dark for 24 h at 25°C. The concentration of solubilized pyrene in micellar phase was determined spectrofluorometrically (F7000, Hitachi High-Tech, Japan) at wavelengths of excitation (λ_{ex}) of 336 nm and emission (λ_{em}) of 350–450 nm.

In Vitro Drug Release. The release of CoQ10 from CoQ10-loaded micelles was inquired by the dialysis bag method (28). A screening for the most suitable release medium was performed. Three milliliters of CoQ10-loaded micelles (amount of CoQ10 was 5 mg) was placed in a dialysis bag (cutoff 8–12 kDa, Solarbio, USA) and was kept in 150 mL of release medium at 37°C, agitated at 100 rpm using an incubating shaker. Two-milliliter samples were drawn at predetermined time intervals, and the same volume was replenished with fresh medium. The amount of released CoQ10 was measured using HPLC. The following release mediums were selected for release studies ($n=3$): (i) 0.5% (w/v) polysorbate 80 (pH 7.4 phosphate-buffered saline, PBS), (ii) 1.0% (w/v) polysorbate 80 (pH 7.4 PBS), (iii) 15% ethanol (pH 7.4 PBS), and (iv) 1.0% sodium dodecyl sulfate (0.15 M NaCl) (29–31).

Stability (Refrigerated, Accelerated, and Long-Term) Studies

The thermal stability of CoQ10-loaded micellar samples was monitored under the refrigerated and accelerated conditions for 6 months. They were stored in the closed desiccators with the constant relative humidity (RH) of $75 \pm 5\%$, maintained by saturated NaCl solution. The desiccators were maintained at 4 ± 2 and $30 \pm 2^\circ\text{C}$ respectively in a thermostat without direct light. The appearance, particle size, and PDI were analyzed at the time of 0, 3, and 6 months after treatment.

The long-term stability of CoQ10-loaded micellar samples was also studied at $25 \pm 2^\circ\text{C}$ by the similar strategy as described above. The samples were stored in the closed desiccators with the constant relative humidity (RH) of $60 \pm 10\%$, maintained by saturated NaNO_2 solution for 0, 3, 6, 9, 12, and 18 months. The stability of samples were examined, where the appearance, the particle size, PDI, the content of CoQ10, and related substance were indicators (27,32).

RESULTS

Solubilization by Micelles

CoQ10 was used as test solute for investigating the solubilization properties of HS15 and polysorbate 80. This drug is poorly soluble in water. The HS15 was found to be superior in CoQ10 solubilization compared with polysorbate 80 (Fig. 1) which was visualized by the slightly higher slopes in the linear graph of solubilizing system. HS15 solubilized more CoQ10 than polysorbate 80, which could be explained by the more hydrophobic acyl chain groups in the micellar structure

Fig. 1. Solubilization of CoQ10 at increasing HS15 and polysorbate 80 concentrations

(31,33,34). Furthermore, HS15 had been reported to improve the bioavailability and reduce the cytotoxicity (35–37). Accordingly, a low amount of HS15 was used as the vehicle for the production of CoQ10.

The Hemolysis of HS15

The hemolysis assay was performed to estimate the biocompatibility of the HS15. The level of hemolysis was compared with that of polysorbate 80, which was a typical surfactant approved by FDA for intravenous administration (38). Hemolysis of HS15 and polysorbate 80 at various times are shown in Fig. 2. HS15 showed much less hemolysis than polysorbate 80 at the concentrations of 0.12 and 0.24 mg/mL. In addition, the hemolysis was 40 and 90% for HS15 and polysorbate 80, respectively, while the concentration increased to 0.24 mg/mL in this study. This indicated that HS15 had much better biocompatibility than polysorbate 80. It could be noted that as the concentration increased from 0.24 to 0.6 mg/mL, hemolysis induced by HS15 increased dramatically from 44.41 ± 0.10 to $99.13 \pm 0.21\%$, but it was lower than that of polysorbate 80 after 24 h. This indicated that the concentration of HS15 in the micelles should be less than 0.60 mg/mL.

Fig. 2. Hemolysis of HS15 and polysorbate 80 in 24 h (Tw 80: polysorbate 80)

from the perspective of safety. Hence, HS15 was excellently used as the vehicle for poorly soluble drugs.

Preparation and Optimization of CoQ10-Loaded Micelles

Effect of the Ratio

In the present study, the amount of CoQ10 was kept constant (5 mg) and HS15 concentration was varied (i.e., 10, 15, 20, 30, 35, and 40 mg). The effect of different weight ratios of CoQ10 and HS15 on the transmittance, particle size, polydispersity index (PDI), entrapment efficiency (EE%), and drug loading (DL%) is summarized in Table I. It became evident from the results—an emulsion with large particle size (>100 nm) and smaller transmittance was obtained when CoQ10 was added alone with HS15 in the ratio of 1:3. This explained by the release of the unencapsulated CoQ10. With increasing of the ratio (from 1:4 to 1:6), decreases in the particles size and PDI were obtained. Further, the ratio was varied to 1:7 and 1:8 (w/w) for fine tuning the formula, but this led to a significant decrease of DL%. Hence, the formulation F-5 (1:6) was picked as the optimized ratio for further studies.

Effect of the Stabilizer

The optimized ratio (1:6) was used to prepare the CoQ10-loaded micelles. We used as stabilizers 0.8% of sodium pyrosulfite, cysteine, thiourea, and glucose (Table II). It showed lower content of the related substance after sterilization compared with that of the others. Hence, glucose was selected in our subsequent formulation. Further, glucose concentrations were varied from 0.05 to 1.0% (w/v) for fine tuning the formula. Table III shows the effect of different concentrations on the content of CoQ10 and the related substance. Increasing the concentration and storage interval, the content of related substance was weakly charged but led to a significant increase of the content of CoQ10 except for 0.2%. Furthermore, the extremum contents of CoQ10 and the related substance were 97.70 and 0.33%, respectively, at 0.20% glucose. Hence, 0.2% (w/v) glucose in the formulation was selected.

Effect of pH

In order to find the optimum preparation method for the CoQ10-loaded micelles, the pH of the solution was adjusted to 3–7 by using HCl or NaOH. Table IV shows the change of particle size, PDI, zeta potential, and the related substance at different pH values. It was clear from the data that no significant changes of the particle size, PDI, zeta potential, and CoQ10 content were observed. Nevertheless, the minimum particle size and content of the related substance after sterilization were 18.95 ± 0.63 nm and 0.26%, respectively, at pH 4. Hence, pH 4 was selected for further optimization studies.

Effect of Sterilization

It was shown by the literature (39) that autoclaving was possible in the case of CoQ10 stabilized injection. The CoQ10-loaded micellar samples were sterilized at 118°C for 25 min or at 121°C for 20 min. After sterilization, the obtained sample

Table I. Effect of the Ratio of CoQ10 and HS15 on the Transmittance, Particle Size, PDI, EE%, and DL%

Batch	Ratio	Transmittance (%)	Particle size (nm)	PDI	EE %	DL %
F-1	1:2	75.5	111.70±1.00	0.223±0.014	–	–
F-2	1:3	75.0	127.00±1.30	0.252±0.009	–	–
F-3	1:4	98.9	24.25±0.56	0.324±0.008	99.96	16.62
F-4	1:5	98.6	20.60±0.46	0.257±0.028	99.91	13.75
F-5	1:6	98.9	20.92±0.43	0.193±0.009	99.39	12.10
F-6	1:7	98.7	19.34±0.23	0.245±0.028	99.85	10.04
F-7	1:8	98.6	17.89±0.15	0.223±0.024	98.75	10.05

PDI polydispersity index, EE% entrapment efficiency, DL% drug loading

and the particle size along with PDI were checked by zeta sizer. Different properties of sterilized CoQ10-loaded micellar samples such as the particle size, PDI, the content of CoQ10, and the related substance (before and after sterilization) are given in Table V. In both cases, the particle size and PDI increased slightly after sterilization. The mean CoQ10 contents after sterilization were found to be 98.47 and 91.32% with the related substance of 0.39 and 4.91%, respectively. This may be due to more degradation of the CoQ10-loaded micellar sample under higher temperature. Hence, 118°C for 25 min was selected for sterilization.

Characterization of Micelles

The Particle Size Analysis by DLS

CoQ10-loaded micellar particle size of the sample was measured by DLS. The mean particle size, PDI, and zeta potential of the sample were found to be 19.76 nm (Fig. 3), 0.112, and –3.405 mV, respectively, by the optimized method and condition.

EE% and DL%

CoQ10 molecule comprises a quinone ring and a long isoprene side chain, which renders it lipophilic and favorable for encapsulation via micelles (40). CoQ10-loaded micelles were in the supernatant, while the unencapsulated CoQ10 likely existed as crystals in the precipitation by centrifugation.

Therefore, it has been proved that unencapsulated CoQ10 was completely removed by centrifugation, so we proceeded to analyze EE% and DL%. Nevertheless, the structure of micelles might be disaggregated by centrifugation. The centrifugal speeds of 14,000 rpm for 5 min was tried in the case of irregular and low “EE%.” With an optimal speed of centrifugation (data not shown), the desired regularity EE% was achieved at 12,000 rpm. Hence, 12,000 rpm was selected for further research. Effect of centrifugal time on EE% and DL% was studied when the centrifugal speed is 12,000 rpm (Fig. 4). Figure 4 shows a significant reduction in EE% with the increasing centrifugal time (0 to 10 min, 15 to 20 min), which could be explained by the release of the unencapsulated CoQ10 and disintegration of CoQ10-load micelles, respectively. Thereby, the EE% and DL% of CoQ10-loaded micelles were 99.39 and 13.77%, respectively, under centrifugal condition of 12,000 rpm for 10–15 min. They were higher than some polymeric micelles due to the nature of HS15 together with a longer hydrophobic block.

Differential Scanning Calorimetry

DSC was performed to further test the physical states of CoQ10-loaded micelles. The DSC thermograms of CoQ10-loaded micelles, HS15, CoQ10, and physical mixture are shown in Fig. 5. The DSC curves of pure CoQ10 and HS15 exhibited endothermic peaks around 54 and 30°C, respectively, which corresponded to their intrinsic melting points. CoQ10-HS15 micelles did not show any melting peak of drug or HS15,

Table II. Effect of Type of Stabilizer on the Content of CoQ10 and the Related Substance

Type	Content (%)	Without sterilization	With sterilization, storage interval (week)			
			0	1	2	3
Sodium metabisulfite	CoQ10	97.89	97.31	96.97	94.97	90.22
	The related substance	0.49	0.66	0.61	0.75	0.82
Cysteine	CoQ10	98.70	95.96	94.76	–	–
	The related substance	0.47	0.98	1.23	–	–
Thiourea	CoQ10	100.00	98.65	95.41	95.59	95.04
	The related substance	0.39	0.53	0.70	0.73	0.79
Glucose	CoQ10	99.17	98.54	98.54	98.01	97.90
	The related substance	0.32	0.32	0.40	0.50	0.54

“–” unmeasured

CoQ10 coenzyme Q10

Table III. Effect of Concentration of Glucose on the Content of CoQ10 and the Related Substance

Content (%)	Concentration of glucose (%)	Without sterilization	With sterilization, storage interval (week)			
			0	1	2	3
CoQ10	0.05	99.54	98.20	97.73	97.60	97.32
	0.10	99.61	99.39	98.44	97.97	97.62
	0.20	99.55	98.83	97.63	96.91	97.70
	0.50	99.83	99.02	98.29	96.27	96.19
	1.00	100.00	98.03	98.46	97.76	97.71
The related substance	0.05	0.15	0.35	0.38	0.43	0.52
	0.10	0.21	0.35	0.42	0.44	0.56
	0.20	0.17	0.33	0.31	0.33	0.33
	0.50	0.19	0.32	0.30	0.42	0.43
	1.00	0.28	0.29	0.40	0.39	0.45

CoQ10 coenzyme Q10

indicating that CoQ10 might have dispersed into molecular form in the carrier. On the contrary, the physical mixture indicated the melting peak of CoQ10 and HS15. Taken together, these DSC profiles demonstrated that the CoQ10-HS15 micelles, composed of HS15 and CoQ10, were not a simple physical mixture of their individual component.

CMC

As an important fluorescence probe with long fluorescent lifetime, pyrene has enough sensitivity to the change of polarity. CMC could be calculated at the point where the I_{373}/I_{384} ratio increases dramatically (41). The graphs for CMC determination of unloaded and CoQ10-loaded micelles are provided in Fig 6. The CMC of unloaded micelles was 5.411×10^{-5} g/mL, which was lower than that of other nonionic surfactants. This result suggested that HS15 could be utilized as an effective means to stabilize chemically unstable drugs as well as to solubilize poorly soluble drug. Significantly, the CMC of CoQ10-loaded micelles was 5.623×10^{-4} g/mL, describing excellent thermodynamic stability of the drug and integrity toward dilution, which was in favor of reducing or avoiding exposure.

In Vitro Drug Release Study

To obtain the best release model and mechanism of CoQ10-loaded micelles, the drug release profile has been

further explored. The minimum solubility of CoQ10 in the medium was 0.17 mg/mL which satisfied the sink condition (>0.1 mg/mL). Figure 7 shows that the highest percentage of released CoQ10 was obtained in 1.0% polysorbate 80, pH 7.4, PBS. In addition, the drug released from micelles started with an initial burst in the first 22.5 h and was followed by a stably sustained release behavior. The cumulative percent of drug released for over 72 h was only 4%. The data of the release over 72 h were fitted using three equations, zero order, first order, and Higuchi equation (Table VI). The obtained results from the kinetic studies are listed in Table VI. These data suggested that the first order was the best fit kinetic model with r^2 value of 0.9843 and 0.9978.

THERMAL STABILITY

The thermal stability of a promising drug candidate plays an important role in the process of drug development. Figure 8 shows the changes in particle size and PDI during the storage time at different temperatures. Upon both 4°C and room temperature, all samples remained more stable compared to those at 30°C. A significant increase in their particle size at 30°C was observed after six months (about 100 nm), and their PDI was up to 0.4, which reflected relatively homogeneous micelles and indicated that the micelles were sensitive to temperature (42).

To identify the storage conditions and handling requirements for the samples and ensure drug quality within the

Table IV. Effect of pH on the Particle Size, PDI, Zeta Potential, the Content of CoQ10, and the Related Substance

pH value	Particle size (nm)	PDI	Zeta potential (mV)	Without sterilization		After sterilization	
				The content of CoQ10 (%)	The content of related substance (%)	The content of CoQ10 (%)	The content of related substance (%)
3	20.65±0.38	0.152±0.011	-2.19±0.827	99.10	0.26	98.83	0.34
4	18.95±0.63	0.140±0.037	-2.66±0.233			98.36	0.28
5	19.85±0.70	0.167±0.030	-2.44±0.205			97.80	0.39
6	19.68±0.39	0.218±0.005	-4.36±0.403			98.73	0.41
7	19.42±0.22	0.175±0.019	-4.46±0.339			98.24	0.44

PDI polydispersity index, *CoQ10* coenzyme Q10

Table V. Effect of Sterilization on the Particle Size, PDI, Zeta Potential, the Content of CoQ10, and the Related Substance

Condition	Particle size (nm)	3PDI	Zeta potential (mV)	The content of CoQ10 (%)	The content of related substance (%)
Before sterilization	18.73±0.27	0.082±0.027	-1.46±0.29	99.27	0.38
118°C, 25 min	19.76±0.13	0.112±0.007	-3.41±1.24	98.47	0.39
121°C, 20 min	19.56±0.03	0.101±0.012	-2.54±0.25	91.32	4.91

PDI polydispersity index, CoQ10 coenzyme Q10

recommended period of validity, three primary batches of samples were further investigated on their stability under accelerated (30°C for 6 months) and long-term (25°C for 18 months) conditions. Apparently, pH, the content of CoQ10, and the related substance of the samples were examined. The assay results are summarized in Table VII. There was no noticeable change on the appearance of sample in the accelerated and long-term stability investigations (data not shown). There was a slight change on pH value of samples. The HPLC results also indicated that the content of CoQ10 and the related substance at 6-month testing periods had more noticeable change as compared to that at 18-month testing periods; in the end, they were charged to 92.93 and 0.74%, respectively. Hence, the CoQ10-loaded micelles had excellent stability below 25°C. Figure 9 shows the regression assay of a drug product with upper and lower acceptance criteria of 105 and 95% of label claim, respectively, according to the regulation of the International Conference of Harmonization (ICH) (40). The lower confidence limit of 95% intersects the lower acceptance criterion of content of CoQ10 at 31.2 months, while the upper confidence limit did not intersect with the upper acceptance criterion until later. The upper one-sided 95% confidence limit for the mean intersects the acceptance criterion of the content of the related substance when it exceeds 36 months. Therefore, the proposed shelf of 24 months can be supported by the statistical analysis of the data.

DISCUSSION

In solubility study, a systematic linear increase in CoQ10 solubility with an increasing concentration of HS15 in water

might be attributed to the improved wettability and dissolution of CoQ10 particles in water by HS15 (8). An indication of the process of transferring CoQ10 from pure water to the aqueous solutions of HS15 was obtained from the change of Gibbs free energy. The Gibbs free energy of transferring (ΔG_{tr}^0) CoQ10 from pure water to the aqueous solutions of HS15 was calculated using Eq. 1, where S_o/S_s is the ratio of molar solubility of CoQ10 in aqueous solutions of HS15 to that of the pure water. The ratio of S_o and S_s is more than 1, so that ΔG_{tr}^0 values were all negative indicating the spontaneous nature of CoQ10 solubilization, and they decreased with an increment in HS15 concentration which demonstrated that the reaction became more favorable as the concentration of HS15 increased.

$$\Delta G_{tr}^0 = -2.303RT \log(S_o/S_s) \quad (1)$$

CoQ10-loaded micelles were prepared by direct dissolution method. It was various process variables like micellar compositions, the preparation condition, and the preparation method that impacted micelles. Formulation F-5 with drug HS15 ratio 1:6 was selected as the optimized ratio because it gave lower particle size, higher entrapment efficiency, and higher drug loading capacity. This might be attributed to the presence of CMC of the CoQ10-loaded micelle which was confirmed by fluorescence probe measurement and was measured to be 5.623×10^{-4} g/mL. The concentration of formulation F-4 was 4.0×10^{-4} g/mL which approached CMC, so that the transmittance of formulation F-4 was much higher than that of formulations F-1, F-2, and F-3. Hence, formulation F-5

Fig. 3. Micellar size distribution of CoQ10-loaded HS15

Fig. 4. Effect of centrifugal time on loading content and entrapment efficiency. The centrifugal speed is 12,000 rpm

Fig. 5. Overlay of DSC thermograms of CoQ₁₀-loaded micelles, HS 15, CoQ₁₀, and physical mixture. (Physical mixture: physical mixture of CoQ₁₀ and HS 15)

was selected as optimized one and taken for further studies concerning pH value, the concentration of stabilizer, and the sterilization condition.

Glucose (0.20%) was found to boast better stability after sterilization with the content of CoQ₁₀ and the related substance standing at 97.70 and 0.33%, respectively. Apart from the above formulation benefits, it was hypothesized that the regular water structure imparted by glucose would assist in enhancing the stability of micelle (43). Optimizing the pH value did not change significantly at particle size, PDI, and zeta potential, but interestingly, stability showed lower content of the related substance (Table IV) due to the microenvironment which was a major factor of micellar stability (14). That could be attributed to enhancing the drug-core interaction when pH value was 4.

The fate of the micelles after injection was mainly influenced by particle size, PDI, and zeta potential. They were important to the micelles for their recognition or nonrecognition

Fig. 6. Fluorescence intensity ratios of I_{373}/I_{384} versus the logarithm of different HS15 and CoQ₁₀-loaded micellar concentration (a: unloaded micelles; b: CoQ₁₀-loaded micelles)

Table VI. Release Kinetics of CoQ₁₀-Loaded Micelles in 1% of Polysorbate 80

Time/h	Release kinetics	Equation	r^2
0–22.5	Zero order	$R=0.1602t+0.2981$	0.9833
	First order	$\ln(100-R)=-0.0016t-0.0029$	0.9843
	Higuchi equation	$R=0.7965t^{1/2}-0.2450$	0.9826
22.5–72.0	Zero order	$R=0.0046t+3.7169$	0.9978
	First order	$\ln(100-R)=0.04115t-0.0379$	0.9978
	Higuchi equation	$R=0.0612t^{1/2}+3.5204$	0.9898

R = percentage of released CoQ₁₀; t = the time of release study

tion by the defense mechanism of body (44). The optimized CoQ₁₀-loaded micelles showed a mean particle size of 19.76 nm. Homogeneous and small particle sizes (<200 nm) could reduce the uptake of the reticuloendothelial system (40). Furthermore, it was expected to enhance the circulation time in blood which in turn could improve the site-specific targeting (38). The mean particle size of blank micelle was 12.41 nm which was smaller than the CoQ₁₀-loaded micelles. One can deduce from those findings that the HS15 was not only incorporated within the micellar core but also possibly was integrated into the micellar shell or was adsorbed on the outer shell leading to transformed micelles (23). On one hand, micelles with greater positive or negative zeta potential values produced greater repulsive forces (39). On the other hand, zeta potential of the micelles significantly influenced the stability of drug in blood; specifically, micelles with lower positive or negative zeta potential values reduced its absorption toward albumin and platelet (40), which also improved the site specific targeting.

The initial fast release phase in Fig. 7 could be attributed to the hydration of the interfacial drug molecules and their passive diffusion. The slow (sustained) release was primarily due to the slow diffusion of drug and the erosion or swelling of micelles as shown in literature (27,45). Common drug release profiles from nanoparticles (with surfactants present) exhibited a short and considerable “burst”

Fig. 7. Release profiles of CoQ₁₀ from CoQ₁₀-loaded micelles in comparison release medium

Fig. 8. The particle size and PDI profiles of CoQ10-loaded micelles in different temperatures

lasting 1–2 days followed by slower, but nearly 100% drug release. Our CoQ10-loaded micelles exhibited a similar profile with an important difference, which resulted from the interactions between the chains in the micellar core and the drug preventing 100% drug release (7,9,31,39,42). Furthermore, the spatial position of a certain drug within a micelle will depend on its polarity. In aqueous systems, nonpolar molecules will be solubilized within the micelle core (46). CoQ10 is a nonpolar molecule, so that CoQ10 in the micelle core compartment. In addition, an increase in the length of a hydrophobic region of surfactant facilitates the solubilization of the hydrophobic drug inside the micelle core (46). HS15 is a nonionic surfactant, and its molecular structure contains long stearic acid chain. We suspect that the drug release may also be influenced by spatial position of a certain drug. This slow burst and possibility of biologically mediated CoQ10 release were exciting indicators for sustained antioxidant drug delivery.

Table VII. Accelerated and Long-Term Stability Results of CoQ10-Loaded Micelles

Storage interval (month)	pH	Content (%)	
		CoQ10	Related substance
Initial	3.98±0.04	100.00±0.56	0.20±0.01
Accelerated stability (6 months, 30±2°C)			
3	3.89±0.07	94.78±0.55	0.53±0.00
6	3.85±0.07	92.93±0.42	0.74±0.03
Long-term stability (18 months, 25±2°C)			
3	3.95±0.04	99.71±0.61	0.24±0.01
6	3.98±0.03	99.08±0.64	0.31±0.02
9	3.97±0.03	98.60±0.56	0.37±0.02
12	4.02±0.03	98.13±0.52	0.44±0.02
18	3.98±0.02	97.37±0.67	0.69±0.01

CoQ10 coenzyme Q10

Fig. 9. Shelf life estimation with upper and lower acceptance criteria based on assay at 25°C

The data of thermal stability study revealed the stability of CoQ10-loaded micelle samples was fine, while the marketed formulation Hefu® was beneficial to crystallization. This could be explained by difference of structure between HS15 and polysorbate 80, and the former has more hydrophobic acyl chain groups than the latter. Owen *et al.* indicated the stability was directly related to the hydrophobic character of the core, which influenced the drug-core interactions (14,47).

CONCLUSION

In the present study, a new surfactant HS15 was investigated as a component of colloidal drug carrier system for CoQ10. Different ratios of HS15/CoQ10, pH value, concentration of glucose, and condition of sterilization were investigated to form a micellar system. It was proved that the concentration of HS15 influenced the particle size, PDI, entrapment efficiency, and drug loading capacity. Furthermore, the pH value and the related substance content were closely linked. The concentration of glucose and the sterilization condition also effects on the stability. In summary, all these characterizations data support the notion that CoQ10-loaded micelle is a useful system for the effect of CoQ10 on cardiovascular health. The osmotic pressure of CoQ10-loaded micelle is 285 mOsmol/kg which suggests that it has good isotonicity. The shelf life of the CoQ10-loaded micellar system can be predicted as 24 months based on the storage stability results. Again, this method could be easily scaled up for commercial purpose. Hence, the results of this study showed that HS15 micelle can be applied as drug carrier systems for many poorly soluble or nonsoluble drugs, especially for CoQ10.

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