J Korean Soc Appl Biol Chem (2012) 55, 619–623
10.1007/s13765-012-2037-1

ORIGINAL ARTICLE

Development of Nanoparticulate Formulation of Coenzyme Q_{10} and Comparison of Plasma Coenzyme Q_{10} Response in a Single Supplementation with Regular Coenzyme Q10 Using Rats

Hyang-Mok Ok · Sang-Mi Kim · Joo-Won Park · Kab-Sig Kim · Han-Kyul Nam · Ji Yeon Kim · Oran Kwon

Received: 21 February 2012 / Accepted: 3 July 2012 / Published Online: 31 October 2012

Abstract Bioavailability of a nanoparticulate formulation of coenzyme Q_{10} (NQ20), which has high stability in the water phase, was evaluated. The particle size was 188 nm, and the zeta potential value was between –38.8 and –44.8 mV at 4, 25, and 40ºC in distilled water after eight weeks storage. Bioavailability of NQ20 was compared with a commercial coenzyme Q_{10} in oil and water phases as emulsified form using male Sprague-Dawley rats. After single oral administration of each coenzyme Q_{10} solution, the blood of rats was collected at 0, 1, 2, 3, 4, 5, 6, 9, and 12 h, and the concentrations of coenzyme Q_{10} were analyzed by high performance liquid chromatography. The plasma coenzyme Q_{10} levels at 1, 2, and 12 h were significantly higher when the rats were administered NQ20 compared to coenzyme Q_{10} in oil. The maximum plasma concentration (C_{max}) and area under the concentration-time curve (AUC) values for NQ20 were 1.10±0.18 and 5.92±1.11 mM·h/mL, whereas the C_{max} and AUC of coenzyme Q_{10} in oil were 0.79±0.07 and 5.30±0.62 mM·h/mL, respectively (p >0.05). Due to high absorbability and small particle size, NQ20 was found to have similar bioavailability to commercial coenzyme Q10 in the oil phase. Results indicate applicability of NQ20 in the food industry, particularly in beverages.

Keywords bioavailability · coenzyme Q_{10} · nanoparticulate · stability

H.-M. Ok · S.-M. Kim · O. Kwon (✉)
Department of Nutritional Science and Food Management, Ewha Womans University, 11-1 Daehyeon-dong, Seodeamun-gu, Seoul 120-750, Republic of Korea
E-mail: orank@ewha.ac.kr

J.-W. Park · K.-S. Kim
Bio-Synetics, 1517 Byucksan Digital Valley II, 481-10, Gasan-dong, Geumchun-Gu, Seoul 153-783, Republic of Korea

H.-K. Nam
Kyungbok Girl's High School, 40 63gil Hwagokro Gangseo-Gu, Seoul 157-733, Republic of Korea

J. Y. Kim (✉)
Department of Food Science and Technology, Seoul National University of Science and Technology, 232 Gongneung-ro, Nowon-gu, Seoul 139-743, Republic of Korea
E-mail: jiyeonk@seoultech.ac.kr

Introduction

Coenzyme Q_{10} (CoQ_{10}) is a yellow-colored crystalline compound, easily soluble in lipid and has a melting point of approximately 50 (Kommuru et al., 2001). Benzoquinone ring structure is a common feature of CoQ_{10} with an isoprenoid side chain (Fig. 1). CoQ_{10} has a low absorption rate from the small intestine, with poor bioavailability in humans (Tomono et al., 1986; Siekmann and Westesen, 1995). Although CoQ_{10} exists in all organisms in the tissue, it decreases progressively after the age of 21 years (Dhanasekaran and Ren, 2005). However, a deficiency of CoQ_{10} has been implicated in several diseases, such as cardiomyopathy, hypertension, angina pectoris, and atherosclerosis. CoQ_{10} functions as an antioxidant including protection of cellular membranes and plasma lipoproteins from free radical-induced damages. Main food sources of CoQ_{10} are meat, poultry, fish, and rapeseed oil (Purchas et al., 2006), and the use of CoQ_{10} in functional foods is very promising, particularly because its properties fit the basic criteria of food fortifier. As a consequence, numerous proprietary preparations of CoQ_{10} are currently available on the market. However, bioavailability of CoQ_{10} in most food products may be very low due to insolubility in water, and showed a low absorption in the gastrointestinal tract (Prosek et al., 2008). Schulz et al.

(2006) reported that a novel soluble formulation of CoQ_{10} (Solu^{TM}Q10) was clearly superior to the oily dispersion and crystalline CoQ_{10} in overall bioavailability. Recently, novel liquid nano-emulsion (NE) and dry-emulsion (DE) formulations were developed to solubilize lipophilic drugs (Hatanaka et al., 2008). NE techniques were often applied to lipophilic drugs, such as paclitaxel (Khandavilli and Panchagnula, 2007), primaquine (Singh and Vingkar, 2008), saquinavir (Vyas et al., 2008), and ramipril (Shafiq et al., 2007). Additionally, DE techniques improved various physicochemical properties of formulations based upon solubility, photostability (Jang et al., 2006), and redispersibility, which improves the bioavailability (Dollo et al., 2003). Importantly, Chopra et al. (1998) showed that the bioavailability of CoQ_{10}, as measured by the area under the concentration-time curve (AUC), varies widely between different oral formulations. Therefore, in the present study CoQ_{10} nanoparticles were developed using supercritical fluid extraction based on the need for improved formulations that are appropriate for food applications and oral bioavailability. To determine the bioavailability of the CoQ_{10} nanoparticulate formulation (NQ20), NQ20 bioavailability was compared with those of commercially available CoQ_{10} preparations in both oil and emulsified phases in rats.

Materials and Methods

Materials. CoQ_{10} was obtained from Zhejiang NHU Co, Ltd. (China). The nanoparticulate coenzyme Q10 in NQ20 was produced by milling raw coenzyme Q10 with fine sugar particles and excipients. NQ20 was emulsified with a sucrose fatty acid ester, poly-glycerin fatty acid ester, and sucrose (Patent number: KR 10-0603974). Soybean oil for the oil suspension was purchased from CJ Cheiljedang (Korea). All other materials and reagents were of analytical grade and used in the state they were received without further purification.

Physical stability. The effective hydrodynamic diameters (D_{eff}) of the particles and the polydispersity index value (PDI) were measured by photon correlation spectroscopy using a "Zetasizer Nano-ZS" (Malvern Instruments, UK) equipped with the Multi Angle Sizing Option (BIMAS). Sizing measurements were performed in a thermostatic cell at a scattering angle of 90º. Zeta potentials, which are surface charge of the nanoparticles, were calculated from the electrophoretic mobilities determined using a "Zetasizer Nano-ZS" (Malvern Instruments). The dispersion was diluted with 1 mL buffer (0.1 μM citric acid and 0.1 μM sodium acetate; pH 3) and 1 mL distilled water in 0.3 mg NQ20, and then sterilized at 90°C for 3 min. We also In addition, stability of the dispersion was assessed by storing for 8 weeks at different temperatures of 4, 25, and 40°C. The measurements were conducted after adjusting each pH at 25°C.

Animals. Thirty 6-week-old male Sprague-Dawley rats weighing 180–200 g were purchased from G-Bio Lab Animal Inc (Korea). The rats were housed in individual cages in an air-conditioned room (22–24°C) with a 12 h light/dark cycle and 45±5% humidity. All animals were provided with a sterile commercial diet and tap water *ad libitum*. The rats were used in the experiments after one week acclimatization. This study was approved by the Institutional Animal Care and Use Committee at Ewha Womans University.

Experimental design for supplemented CoQ_{10}. The study consisted of a single-dose comparative bioavailability analysis of three CoQ_{10} preparations with a 7-day washout period between treatments. The rats were randomly assigned into three groups (n=9). NQ20 and emulsified CoQ_{10} were mixed with water, and regular CoQ_{10} was dissolved in soybean oil. Rats received CoQ_{10} at 60 mg/kg using a feeding needle, and venous blood samples were collected at 0, 1, 2, 3, 4, 5, 6, 9, and 12 h after administration. Blood was collected in EDTA-treated tubes and rapidly centrifuged at 4°C for 10 min at 3,000×g to obtain plasma for high performance liquid chromatography (HPLC). The plasma was immediately frozen at −20°C, and all tubes were wrapped with aluminum foil to prevent light exposure.

Analysis of plasma concentrations of coenzyme Q_{10} after oral administration. An internal standard (5 μL) (CoQ_9, 79.5 μg/mL in hexane) was added to 200 μL of plasma and mixed by vortexing. The plasma was then mixed with 1 mL of 1-propanol, mixed by vortexing for 5 min, and centrifuged at 3000×g for 5 min to precipitate the proteins. The supernatant (1 mL) was evaporated at 25°C under a nitrogen gas stream. The residue was reconstituted with 100 μL of ethanol, and 20 μL of the resulting solution was analyzed by HPLC. The plasma concentration-time profile was corrected for endogenous levels of CoQ_{10} as follows. For each animal, the respective endogenous levels of CoQ_{10} at time 0 h were subtracted from the observed CoQ_{10} concentrations at each time point. The concentrations of CoQ_{10} were determined using an HPLC system (Peekman SP Co, Ltd., Korea) consisting of a UV detector (SP3002), a pump (SP3101), and an automatic injector (SP3023). The wavelength of the UV detector was set at 275 nm, and an octadecylsilane column (C18, 3 mm × 250 mm, 5 μm; Shiseido, Japan) was eluted with mobile phase at a flow rate of 0.3 mL/min. The mobile phase was ethanol (HPLC grade, J.T. Baker, USA). The column was maintained at 30°C in a column oven. A 20 μL volume of plasma was injected, and the detector output was recorded using a data management system (Ezchrom quick soft version 3.1, Shimadzu, Japan).

Statistical analysis. The AUC was calculated using the linear trapezoidal rule from zero to the last plasma concentration. The peak plasma concentration (C_{max}) and the time to reach peak plasma concentration (T_{max}) were calculated from the experimental data. Non-compartmental analysis was performed using K-BE 2007.7 version 1.0.0 (Korea Food & Drug Administration, Korea). The plasma CoQ_{10} concentrations for each CoQ_{10} preparation were analyzed with a general repeated-measures linear model. Analysis of variance (ANOVA) was then performed to test each variable at individual time points with *post-hoc* Duncan multiple comparison tests. Significance was set at $p < 0.05$ for all statistical analyses. Data are expressed as means ± standard error (SE). All

Table 1 Particle size and zeta-potential of the nanoparticulate formulation of coenzyme Q_{10} (NQ20) in different solvents during storage over eight weeks

Medium	Particle size (nm)									Zeta potential (mV)		
Storage Period (wk)	0 wks			2 wks			8 wks			8 wks		
Temperature (°C)	4	25	40	4	25	40	4	25	40	4	25	40
NQ20 in pH 3 buffer	188	188	188	220	224	224	220	226	219	-4.82	-5.95	-13.9
NQ20 in water	188	188	188	195	193	197	197	200	203	-44.8	-38.8	-44.4

The data shown are means.

statistical analyses were performed using the Statistical Analysis Systems package version 9.2 (SAS Institute, USA).

Results and Discussion

Physicochemical characteristics of NQ20. Particle sizes of NQ20 were analyzed under different temperature and pH conditions over eight weeks to characterize NQ20. NQ20 was soluble in water and in pH 3 buffer without any heating or sonication. The initial average particle size of NQ20 was 188 nm (Table 1). The nanoparticles in distilled water increased slightly to 203 nm over time at 4, 25, and 40°C. However, the particle size in water was much smaller than that in the pH 3 buffer over eight weeks at all three temperatures. The polydispersity index (PdI), which describes the width of the particle size distribution of NQ20 in pH 3 buffer, slowly increased from 0.168 to 0.337 over time at 4, 25, and 40°C. Moreover, the PdI of NQ20 in water also increased from 0.168 to 0.204 over time at 4, 25, and 40°C (data not shown). In general, nano-emulsions are fine oil-in-water dispersions that have droplets of approximately 100–600 nm in size (Bouchemal et al, 2004). Our nanoparticles were also within this range. A nano-emulsion should be stable in aqueous solution, because the rate of Brownian motion is much faster than the rate of creaming and sedimentation (Mosqueira et al., 2000; Sarker, 2005). Colloidal suspended particles carry an electrical charge, and because the zeta potential is a good index of the magnitude of the interaction between colloidal particles, its measurements are commonly used to assess the stability of colloidal systems. The measurement technique used by the Zetasizer Nano-ZS to measure the zeta potential of particles in a solution is known as phase analysis light scattering. This technique uses a laser, which passes through the sample, to measure the velocity of the particles in an applied electric field of a known value (Bihari et al, 2008). The average zeta potential value of NQ20 in distilled water at 4, 25, and 40°C over eight weeks was –42.7 mV without any creaming or aggregation (Table 1). In contrast, the average zeta potential at all temperatures in pH 3 buffer was –8.2 mV. Although only the representative results for physicochemical characteristics were used, zeta potential still provides an important indication for the stability of colloidal system (Mady and Darwish, 2010). Particles with zeta potentials that are more positive than +30 mV or more negative than –30 mV are generally considered as stable (Gallardo et al, 2005). In addition, zeta potentials above (+/–) 30 mV has been shown to be stable in suspension, as the surface charge prevents aggregation of the particles (Mohanraj and Chen, 2006). In addition, the zeta potential of the particles has been known to be depended on the pH and the electrolyte concentration of the sample solution system (Illés and Tombácz, 2006). In our study, higher physical stability of NQ20 in the aqueous system can be attributed to the smaller particle size. Therefore, distilled water, which has neutral pH, was a better medium for NQ20 stability than the acidic buffer.

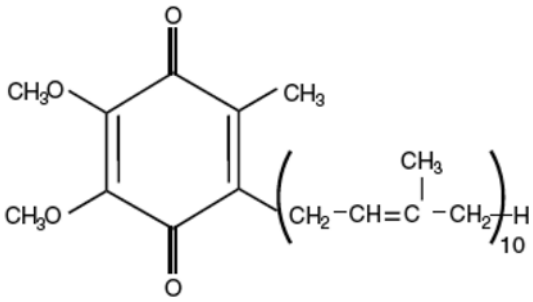

Fig. 1 Structure of coenzyme Q_{10}. Coenzyme Q (CoQ, ubiquinone) belongs to a homologous series of compounds that share a common benzoquinone ring structure with isoprenoid side chains of varying lengths.

Comparison of bioavailability of NQ20 with Regular CoQ_{10}. Under normal circumstances, plasma CoQ_{10} concentrations are not substantially affected by dietary components, such as dairy products, eggs, fish, and vegetables (Kaikkonen et al., 1999). CoQ_{10} supplementation, on the other hand, leads to the increase in plasma CoQ_{10} concentration, the extent of which depends on the dosage, duration, and type of formulation. There are numerous reports in the literature on plasma CoQ_{10} response to oral administration of CoQ_{10} in several species of animals as well as humans (Bhagavan and Chora, 2006; Kaikkonen et al., 2002; Miles et al., 2002); The oral bioavailability of the NQ20 product was investigated and compared to that of a regular CoQ_{10} in oil and in an emulsified product using Sprague-Dawley rats. The baseline plasma CoQ_{10} concentration (mean ± SE) of all groups was 0.081±0.011 μM/mL. The increase in the mean plasma CoQ_{10} concentration versus the time profile for all three CoQ_{10} formulations is presented in Fig. 2. Although the plasma CoQ_{10} concentrations did not differ over time by repeated-measures ANOVA (p=0.0523), a significantly higher plasma CoQ_{10} concentrations was observed when the rats were administered NQ20 at each time point comparing the emulsified CoQ_{10}, with

Table 2 Pharmacokinetic parameters in rats following oral administration of CoQ_{10} (60 mg/kg) in three different formulations (mean ± SE, n =9)[a]

Group	C_{max} (μM/mL)	T_{max} (h)	AUC (μM·h/mL)
NQ20	1.10±0.18[a]	5.2±1.36[ab]	5.92±1.11[a]
Emulsified CoQ_{10}	0.42±0.06[b]	8.4±1.14[a]	3.04±0.47[b]
CoQ_{10} in oil	0.79±0.07[a]	4.3±1.05[b]	5.30±0.62[ab]

[a]Values are mean ± standard error.
[b]Values with different letters are significantly different at α=0.05 levels by Duncan's multiple range test (n =9)

the exception of the 9-h time point (p <0.05). The curves for the formulations were quite similar with regard to the time to peak plasma concentration, whereas an additional smaller peak was observed at 12 h. In contrast, the emulsified CoQ_{10} showed a slightly different peak pattern after administration, where the plasma concentration tended to increase steadily after administration of the emulsified CoQ_{10} over 12 h. Among these three formulas, the emulsified CoQ_{10} had the lowest plasma CoQ_{10} concentrations over time. Although administration of NQ20 resulted in slightly higher plasma levels than those of CoQ_{10} in oil, no significant difference was observed. The results of AUC, C_{max}, and T_{max} parameters are summarized in Table 2. The mean AUCs (±SE) were 5.92±1.11, 5.30±0.62, and 3.04±0.47 μM·h/mL for NQ20, CoQ_{10} in oil, and emulsified CoQ_{10}, respectively. Importantly, the AUC of NQ20 was significantly higher than that of emulsified CoQ_{10} (p <0.05); however, no significant differences were observed between that of NQ20 and CoQ_{10} in oil. T_{max} of NQ20 was approximately 5.2 h after administration and that of CoQ_{10} in oil was approximately 4.3 h; however, T_{max} of the emulsified CoQ_{10} reached a peak at approximately 8.4 h (p <0.05). The time to reach maximum (T_{max}) was 1.6-fold faster in NQ20 than that of emulsified CoQ_{10}. Therefore, the T_{max} of NQ20 indicated a more rapid absorption of CoQ_{10} as compared with the emulsified CoQ_{10}, though the difference was not statistically significant. A significant difference (p <0.05) in change of plasma CoQ_{10} concentrations was found until 5 h between NQ20 and the emulsified CoQ_{10}, indicating that oral absorption of CoQ_{10} was enhanced by the particle size reduction. While the emulsified CoQ_{10} slowly increased until 8 h in the plasma concentration of $CoQ_{10,}$ NQ20 increased fast until 5 h after NQ20 administration. There was, therefore, no significant difference after 6 h between the two groups. However, with slow absorption of emulsified CoQ10, the AUC value was 1.9-fold lower than that of NQ20. A relatively slow absorption of CoQ10 of the emulsified CoQ_{10} indicates that CoQ_{10} is absorbed slowly from gastrointestinal tract and this may be attributable to low water solubility. NQ20 in aqueous system made rapid absorption possible with bile acid in the intestinal tract by the intestinal mucosal layer. The maximum plasma concentrations (C_{max}) of NQ20 and CoQ_{10} in oil were 1.10±0.18 and 0.79±0.07 μM/mL, respectively, which were significantly higher than those in rats administered the emulsified CoQ_{10} (0.42±0.06 μM/mL).

The plasma concentration of total CoQ_{10} in our rat models

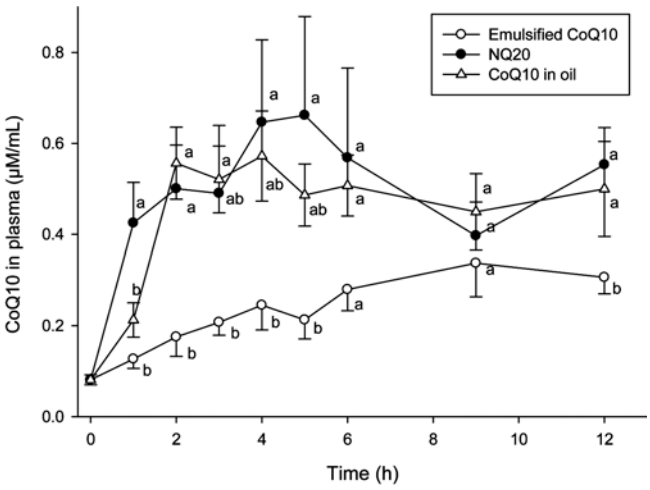

Fig. 2 Changes in plasma CoQ_{10} concentrations in rats after a single oral administration of each CoQ_{10} preparation. Rats were orally administered a single dose of 60 mg/kg of each CoQ_{10} formulation. Values represent mean ± SE (n =9). For any given time point, the values that do not share the same letter are significantly different (ANOVA, *post-hoc* Duncan test, p <0.05), ○ emulsified CoQ; ● NQ20; △ CoQ_{10} in oil

peaked at 5 h after NQ20 administration. However, in another study, serum CoQ_{10} peaked at 6 h after administration (Nukui et al., 2007), which may have resulted simply from the difference in CoQ_{10} content, since the CoQ_{10} content in that study was twice as high as the concentration used in our study. In contrast to humans, the major form of CoQ in rats is CoQ_9, and therefore exogenously administered CoQ_{10} may be absorbed without being metabolized. Both CoQ_9 and CoQ_{10} are widely distributed in the spleen, kidneys, heart, liver, and plasma. In the present study, a second peak in plasma CoQ_{10} concentration was seen at 12 h after CoQ_{10} administration (Fig. 2). This was attributed to enterohepatic circulation of exogenous CoQ_{10} uptake by the small intestine, followed by secretion of endogenous CoQ_{10} synthesized in the liver into plasma. A 12-h observation period was applied after each administration, similar to several other studies (Joshi et al., 2003; Kurowska et al., 2003; Schulz et al., 2006). In contrast, serum CoQ_{10} levels peaked at 6 h after intake, but peaked again at 24 h after intake in a study, in which deuterium-labeled CoQ_{10} was administered to humans (Tomono et al., 1986), suggesting that a similar mechanism took place for the second peak observed in the current study. However, in our study, the plasma CoQ_{10} level did not completely reach the baseline during the observed time. In our oral administration study over a 12-h period, the peak plasma levels of CoQ_{10} and the elimination phase were not detected after oral administration of the three formulations (Fig. 2). In the case of all formulations, the plasma CoQ10 concentrations at 12 h after dosing increased again without evidence of an elimination phase. Therefore, it was difficult to calculate the half-life of exogenous CoQ_{10} in the present study. Bhagavan (2006) reported a T_{max} of 6.5 h and an elimination half-life of 33.19 h using deuterium-labeled CoQ_{10}. In order to figure out half life of this formulation

(NQ20), the bioavailability test with a longer observation period should be conducted in future studies.

Reducing particle size is a desirable way to improve CoQ_{10} bioavailability. Due to the high absorbability and small particle size, NQ20 shows similar bioavailability in the water and oil phases. These findings indicate that the nanoparticulate formulation of CoQ_{10} in the water phase can be quickly absorbed and maintained by the body as regular CoQ_{10} in an oil phase. These results suggest the applicability of NQ20 in a wide range of beverages in the food industry. In addition to particle size, several factors may also contribute to improvements in oral bioavailability using these strategies, including the capability of spreading to the aqueous phase in the gastrointestinal tract, permeability of the intestinal membrane to substances, stability in the stomach, and gastric empting rate (Hatanaka et al., 2008). Because several parameters govern nanoparticle characteristics, such as surfactant concentration, external phase volume, and the droplet size reduction method, these factors should be well-controlled in the future study.

In the present study, a pharmacokinetics study of CoQ_{10} was undertaken and found that CoQ_{10} exhibits nonlinear kinetics, which may have been caused by the saturation of protein binding in the blood, as shown in a previous study (Nishimura et al., 2009). Importantly, the nanoparticulate formulation of NQ20 achieved a higher plasma concentration of CoQ_{10} compared to the general and emulsified CoQ_{10}.

Acknowledgments This project was supported by the Ministry of Knowledge & Economy (RITD program, Project No. 70004683) and the Ministry of Education, Science, and Technology (Brain Korea 21, Project No. 2006-0519-4-7).

References

Bhagavan HN and Chora RK (2006) Coenzyme Q10: Absorption, tissue uptake, metabolism and pharmacokinetics. *Free Radic Res* **40,** 445–53.

Bihari P, Vippola M, Schultes S, Praetner M, Khandoga AG, Reichel CA et al. (2008) Optimized dispersion of nanoparticles for biological *in vitro* and *in vivo* studies. *Part Fibre Toxicol* **5,** 14.

Bouchemal K, Briançon S, Perrier E, and Fessi H (2004) Nano-emulsion formulation using spontaneous emulsification: solvent, oil and surfactant optimisation. *Int J Pharm* **280,** 241–51.

Chopra RK, Goldman R, Sinatra ST, and Bhagavan HN (1998) Relative bioavailability of coenzyme Q10 formulations in human subjects. *Int J Vitam Nutr Res* **68,** 109–13.

Dhanasekaran M and Ren J (2005) The emerging role of coenzyme Q-10 in aging, neurodegeneration, cardiovascular disease, cancer and diabetes mellitus. *Curr Neurovasc Res* **2,** 447–59.

Dollo G, Le Corre P, Guérin A, Chevanne F, Burgot JL, and Leverge R (2003) Spray-dried redispersible oil-in-water emulsion to improve oral bioavailability of poorly soluble drugs. *Eur J Pharm Sci* **19,** 273–80.

Gallardo V, Morales ME, Ruiz MA, and Delgado AV (2005) An experimental investigation of the stability of ethylcellulose latex: correlation between zeta potential and sedimentation. *Eur J Pharm Sci* **26,** 170–5.

Hatanaka J, Kimura Y, Lai-Fu Z, Onoue S, and Yamada S (2008) Physicochemical and pharmacokinetic characterization of water-soluble Coenzyme Q(10) formulations. *Int J Pharm* **363,** 112–7.

Illés E and Tombácz E (2006) The effect of humic acid adsorption on pH-dependent surface charging and aggregation of magnetite nanoparticles. *J Colloid Interface Sci* **295,** 115–23.

Jang DJ, Jeong EJ, Lee HM, Kim BC, Lim SJ, and Kim CK (2006) Improvement of bioavailability and photostability of amlodipine using redispersible dry emulsion. *Eur J Pharm Sci* **28,** 405–11.

Joshi SS, Sawant SV, Shedge A, and Halpner AD (2003) Comparative bioavailability of two novel coenzyme Q10 preparations in humans. *Int J Clin Pharmacol Ther* **41,** 42–8.

Kaikkonen J, Nyyssönen K, Tuomainen TP, Ristonmaa U, and Salonen JT (1999) Determinants of plasma coenzyme Q10 in humans. *FEBS Lett* **443,** 163–6.

Kaikkonen J, Tuomainen TP, Nyyssonen K, and Salonen JT (2002) Coenzyme Q10: absorption, antioxidative properties, determinants, and plasma levels. *Free Radic Res* **36,** 389–97.

Khandavilli S and Panchagnula R (2007) Nanoemulsions as versatile formulations for paclitaxel delivery: peroral and dermal delivery studies in rats. *J Invest Dermatol* **127,** 154–62.

Kommuru TR, Gurley B, Khan MA, and Reddy IK (2001) Self-emulsifying drug delivery systems (SEDDS) of coenzyme Q10: formulation development and bioavailability assessment. *Int J Pharm* **212,** 233–46.

Kurowska EM, Dresser G, Deutsch L, Bassoo E, and Freeman DJ (2003) Relative bioavailability and antioxidant potential of two coenzyme q10 preparations. *Ann Nutr Metab* **47,** 16–21.

Mady MM and Darwish MM (2010) Effect of chitosan coating on the characteristics of DPPC liposomes *J Adv Res* **1**, 187–91.

Miles M, Horn P, Miles L, Tang P, Steele P, and DeGrauw T (2002) Bioequivalence of coenzyme Q10 from over-the-counter supplements. *Nutr Res* **22**, 919–29.

Mohanraj VJ and ChenY (2006) Nanoparticles- a review. *Trop J Pharm Res* **5,** 561–73.

Mosqueira VC, Legrand P, Pinto-Alphandary H, Puisieux F, and Barratt G (2000) Poly(D,L-lactide) nanocapsules prepared by a solvent displacement process: influence of the composition on physicochemical and structural properties. *J Pharm Sci* **89,** 614–26.

Nishimura A, Yanagawa H, Fujikawa N, Akiko K, and Nobuhito S (2009) Pharmacokinetic Profiles of Coenzyme Q10: Absorption of Three Different Oral Formulations in Rats. *J Health Sci* **55**, 540–8.

Nukui K, Yamagishi T, Miyawaki H, Kettawan A, Okamoto T, and Sato K (2007) Comparison of uptake between PureSorb-Q40 and regular hydrophobic coenzyme Q10 in rats and humans after single oral intake. *J Nutr Sci Vitaminol (Tokyo)* **53,** 187–90.

Prosek M, Butinar J, Lukanc B, Fir MM, Milivojevic L, Krizman M et al. (2008) Bioavailability of water-soluble CoQ10 in beagle dogs. *J Pharm Biomed Anal* **47,** 918–22.

Purchas RW, Busboom JR, and Wilkinson BH (2006) Changes in the forms of iron and in concentrations of taurine, carnosine, coenzyme Q(10), and creatine in beef longissimus muscle with cooking and simulated stomach and duodenal digestion. *Meat Sci* **74,** 443–9.

Sarker DK (2005) Engineering of nanoemulsions for drug delivery. *Curr Drug Deliv* **2,** 297–310.

Schulz C, Obermüller-Jevic UC, Hasselwander O, Bernhardt J, and Biesalski HK (2006) Comparison of the relative bioavailability of different coenzyme Q10 formulations with a novel solubilizate (Solu Q10). *Int J Food Sci Nutr* **57,** 546–55.

Shafiq S, Shakeel F, Talegaonkar S, Ahmad FJ, Khar RK, and Ali M (2007) Development and bioavailability assessment of ramipril nanoemulsion formulation. *Eur J Pharm Biopharm* **66,** 227–43.

Siekmann B and Westesen K (1995) Preparation and physicochemical characterization of aqueous dispersions of coenzyme Q10 nanoparticles. *Pharm Res* **12,** 201–8.

Singh KK and Vingkar SK (2008) Formulation, antimalarial activity and biodistribution of oral lipid nanoemulsion of primaquine. *Int J Pharm* **347,** 136–43.

Tomono Y, Hasegawa J, Seki T, Motegi K, and Morishita N (1986) Pharmacokinetic study of deuterium-labelled coenzyme Q10 in man. *Int J Clin Pharmacol Ther Toxicol* **24,** 536–41.

Vyas TK, Shahiwala A, and Amiji MM (2008) Improved oral bioavailability and brain transport of Saquinavir upon administration in novel nanoemulsion formulations. *Int J Pharm* **347,** 93–101.