Stability and Bioequivalence Studies of Two Marketed Formulations of Coenzyme Q_{10} in Beagle Dogs

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Coenzyme Q_{10} (CoQ$_{10}$), a highly lipophilic compound present in the inner mitochondrial membrane, is essential for production of cellular energy in the form of ATP. CoQ$_{10}$ is used as an antioxidant and also in the treatment of various cardiovascular disorders. The relative bioavailabilities of powder filled capsule (I) and oil-based formulation (II) of CoQ$_{10}$ were compared in beagle dogs in an open, randomized, multiple dose, cross-over design. Poor and slow absorption characteristics were observed for both the formulations. The *AUC*, C_{max}, and T_{max} for formulation I and II are comparable ($p<0.05$) where the values for formulation I are $22.84\pm6.3\,\mu g\,ml^{-1}\,h$, $0.51\pm0.11\,\mu g/ml$, and $6.1\pm2.0\,h$ whereas the values for formulaion II are $24.32\pm5.6\,\mu g\,ml^{-1}\,h$, $0.55\pm0.16\,\mu g/ml$, and $6.6\pm2.3\,h$, respectively. Stability of CoQ$_{10}$ at various temperature and humidity conditions and its photostability were studied. Various antioxidants were evaluated to determine the type and amount of antioxidant(s) required to improve the stability of CoQ$_{10}$. Large extent of degradation was observed at 45°C and 55°C. The effect of humidity conditions on degradation was insignificant. Among the various antioxidants studied, mixture of ascorbic acid (5%) and EDTA (0.1%) offered better protection than phenolic antioxidants such as butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), or propyl gallate (PG). Further, increasing concentrations of phenolic antioxidants (from 0.1 to 0.3%) accelerated the degradation.

Key words coezyme Q_{10}; stability; bioavailability; antioxidants

Coenzyme Q_{10} (CoQ$_{10}$), also known as ubiquinone (Fig.1), is a highly lipophilic compound present in the inner mitochondrial membrane where it acts as an integral part of oxidative phosphorylation.[1] CoQ$_{10}$ is essential for production of cellular energy in the form of ATP. CoQ$_{10}$ is recommended as an antioxidant and also in the treatment of various cardiovascular disorders such as angina pectoris, hypertension, and congestive heart failure.[1] It is poorly and slowly absorbed from gastrointestinal tract due to its high molecular weight and poor aqueous solubility.[2] Currently, CoQ$_{10}$ is available in the market as nutritional supplement in oil-based and powder filled capsule formulations. The oral bioavailability of these formulations, however, differ widely.[3] Only a few studies have reported the comparisons of bioavailabilities of different CoQ$_{10}$ formulations. Kishi *et al.* have reported higher bioavailability for soyabean oil suspension of CoQ$_{10}$ compared to other formulations such as plain tablets, granules, and sugar-coated tablets.[3] Weis *et al.* have reported that soyabean oil suspension of CoQ$_{10}$ without any emulsifiers had higher bioavailability compared to similar formulations containing polysorbate 80 and/or lecithin.[4] However, Chopra *et al.* have reported higher bioavailability for the new solubilized formulation of CoQ$_{10}$ containing emulsifiers compared to the oil suspension and powder filled capsule formulations.[5]

CoQ$_{10}$ is yellow or orange crystalline powder and upon exposure to light, it gradually decomposes and changes its color to dark yellow.[6] Solution preparations of CoQ$_{10}$ are far more prone to photo degradation than in the solid state. Color darkening of the crystals was attributed to change in the surface condition in addition to the structural changes of CoQ$_{10}$ molecules. Further, these physicochemical changes affected the UV absorption properties of solid-state samples.[6] Antioxidants are usually added to improve the stability of

drug formulations. Antioxidants, when properly used before auto-oxidation sets in, are extremely effective in reducing the initial rate of peroxide build up and hence considerably extend the induction period of the oxidation cycle.[7] The choice of antioxidant(s) will depend on the requirements of the system, *i.e.*, high antioxidant potency at a minimal concentration.[7] The amount of antioxidant needed is usually determined empirically. In many cases, the more antioxidant added, the more protection it offers; for others, acceleration of oxidation can occur at high antioxidant concentration.[7] The objectives of the present study are (1) to compare the bioavailability of two marketed (powder filled capsule and an oil-based) formulations of CoQ$_{10}$ in beagle dogs; (2) to study the stability of CoQ$_{10}$ at various temperature, humidity, and light conditions; and (3) to determine the type and amount of antioxidant(s) required to maintain the stability of CoQ$_{10}$ during its shelf life.

Materials and Methods

1. Materials Standard CoQ$_{10}$ was purchased from Spectrum Chemicals (Gardena, CA). The internal standard, CoQ$_9$ was kindly supplied by Eisai Co. (Tokyo, Japan). Sep-Pak silica (100 mg) solid phase extraction cartridges were purchased from Waters (Milford, MA). Antioxidants were purchased from Fluka (Ronkonkoma, NY), and all other chemicals and solvents were purchased from Fisher Scientific (Fair Lawn, NJ).

2. Bioavailability Studies 2.1. Formulations: Two marketed formulations of CoQ$_{10}$ are selected. Formulation I is a powder filled capsule and Formulation II is an essential oil based solubilized formulation. Both the formu-
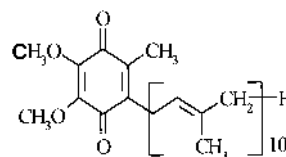

Fig. 1. Structure of Coenzyme Q_{10}

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lations contained 30 mg of CoQ_{10} per capsule.

2.2. Study Protocol: The study was open, randomized, multiple-dose, cross over design. Since CoQ_{10} is poorly absorbed from gastrointestinal tract, it is necessary to administer multiple doses for a few days to raise the plasma concentrations to quantifiable levels and also to facilitate the comparisons of the formulations. Eight male beagle dogs received each of the two formulations on separate occasions. In the first part of the study, blood samples were collected from all the animals prior to dosing to determine the endogenous levels of CoQ_{10}. Four dogs were randomly assigned to each of the two groups. Each dog in the first group received formulation I and the other group received formulation II twice daily at 8 : 00 a.m. and 8 : 00 p.m. for four days. On fifth day, following another dose at 8 : 00 a.m., blood samples were collected from jugular vein into heparinized tubes at 1.0, 2.0, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 24.0, 48.0, and 72.0 h time intervals. After centrifugation, plasma was separated into amber colored vials and stored at $-20\,°C$ until further analysis. Following a seven-day wash out period, the two groups were crossed-over to carry out the second part of the study. The dosing and sampling procedures were identical in these two studies.

2.3. Analysis of Plasma Samples: Plasma concentrations of CoQ_{10} were determined according to our earlier reported method.[8] Since the solutions of CoQ_{10} are susceptible to photodegradation, studies were carried out under the illumination of yellow light. All the containers used were wrapped with aluminum foil. To a milliliter of plasma, 50 μl of internal standard (CoQ_9, 7.5 mg/l in hexane) were added and vortex mixed. The plasma was then deproteinized with 1.0 ml of 10% trichloroacetic acid. To this sample, 2 ml of hexane was added, vortex mixed for 5 min, and centrifuged at $2000 \times \boldsymbol{g}$ for 5 min. The hexane phase was transferred to 100 mg silica-solid phase extraction cartridge, previously activated with 5 ml of hexane and mounted on vacuum manifold system. The extraction was repeated twice in similar fashion and the hexane layer was collected on silica cartridge. The total volume of hexane (6.0 ml) was passed through the cartridge. The cartridge was dried under vacuum for 2 min and eluted with 0.75 ml of methanol : hexane (85 : 15 v/v) mixture. A 100 μl volume of the eluted fraction was injected to the RP-HPLC column (C-18) for separation and UV detection.

2.4. Chromatography: The LC system consisted of Isco-2350 pump, Isco-V^4 variable absorbance detector. The eluate was monitored for absorbance at 275 nm and the detector output was recorded using Isco-Chemresearch (Ver 2.4) Software. A sample of 100 μl volume was injected onto a reversed phase column (Nova-Pak C18, 4μ, 150×3.9 mm i.d., Waters, Milford, MA) preceded by a guard column (Alpha Bond C18, 7.5×4.6 mm i.d., Alltech, San Jose, CA). The isocratic mobile phase was methanol : n-hexane (98 : 2 v/v). The flow rate was $1.0\,ml \cdot min^{-1}$.

2.5. Data Analysis: 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 are subtracted from the observed CoQ_{10} concentrations at each time point. The area under the curve, AUC, was calculated by linear trapezoidal rule from zero to the last plasma concentration. The maximum plasma concentration, C_{max}, and the time of its occurrence, T_{max}, were compiled from the concentration–time data. Analysis of variance (ANOVA) and t-tests were performed to evaluate significant differences between the two formulations. Values are reported mean$\pm$S.D. and the data were considered statistically significant at $p<0.05$.

3.   Stability Studies   Approximately 750 mg of CoQ_{10} was placed in each of several glass vials and were kept at various conditions such as 25 °C, 37 °C, 45 °C, 55 °C, 37 °C-11% RH, 37 °C-51% RH, and 37 °C-91% RH. These vials were wrapped with aluminum foil to prevent exposure to light. Mixtures of CoQ_{10} with various antioxidants were taken in transparent glass vials and were exposed to fluorescent light (600 FC) at 25 °C in a light stability chamber (Model #352643, HotPack Inc., Philadelphia, PA). Another set of samples of CoQ_{10} with various antioxidants were kept at 55 °C and these containers were wrapped in aluminum foil to prevent exposure to light. The antioxidants employed in these studies include ascorbic acid (AA), butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), propyl gallate (PG), and ethylenediaminetetraacetic acid (EDTA).

3.1. Analysis of Stability Samples: Approximately 50 mg of CoQ_{10} were accurately weighed into 100 ml volumetric flask, dissolved in n-hexane, and made up to volume with n-hexane. Three milliliters of the hexane solution was further diluted to 50 ml with methanol. A 50 μl of the methanolic solution was injected into the HPLC. The chromatographic conditions are the same as described in section 2.4. except with a flow rate of 1.5 ml per minutes.

Table 1.   Parameters of the Bioequivalence Studies of CoQ_{10} Formulations

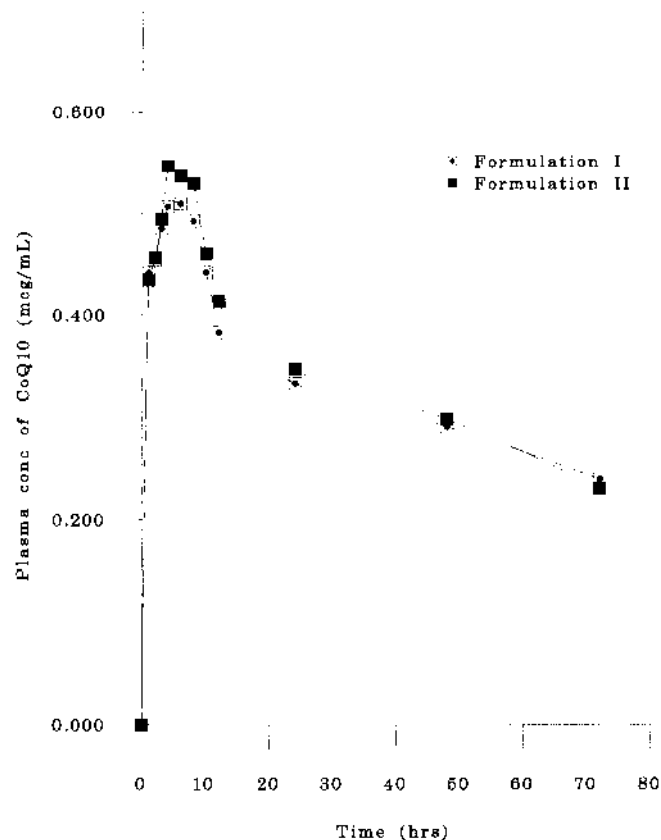| Pharmacokinetic parameter	Formulation I	Formulation II
AUC ($\mu g\,ml^{-1}$ h)	22.84 ± 6.3	24.32 ± 5.6
C_{max} ($\mu g/ml$)	0.51 ± 0.11	0.55 ± 0.16
T_{max} (h)	6.1 ± 2.0	6.6 ± 2.3

Fig. 2.   Mean Plasma Concentration and Time Profiles of CoQ_{10} Formulations

Results and Discussion

Bioavailability Studies   The endogenous plasma levels of CoQ_{10} were determined in the animals of the two study groups and were found to be 0.20 ± 0.06 and $0.22\pm0.05\,\mu g/ml$, respectively. The plasma concentration–time profiles of formulation I and II, calculated by subtracting the endogenous levels of CoQ_{10} at each time point, are shown in Fig. 2. The pharmacokinetic parameters are summarized in Table 1. The AUC, C_{max}, and T_{max} values of formulation I and II are comparable ($p<0.05$) and therefore, the powder filled capsule and oil based formulation are bioequivalent. These results are in agreement with the study reported by Kaikkonen *et al.* In which no significant differences were found between the bioavailabilities of granular and oil-based formulation in humans.[9] Also, Bogentoft *et al.* have reported no significant differences in the bioavailabilities of solid dosage form (tablet) and soyabean oil (soft gelatin capsule) formulation of CoQ_{10}.[10] In a study using deuterium labeled CoQ_{10} in humans, Tomono *et al.* have reported that CoQ_{10} undergoes enterohepatic recycling and thereby increasing the plasma CoQ_{10} levels 24 h after oral administration.[11] In the present investigations, however, no such rise in plasma concentra-
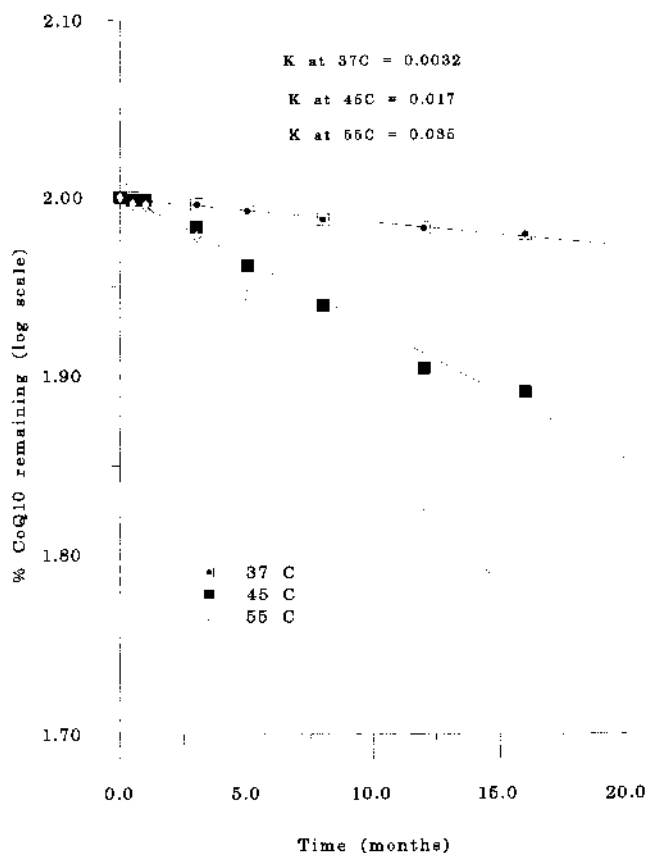
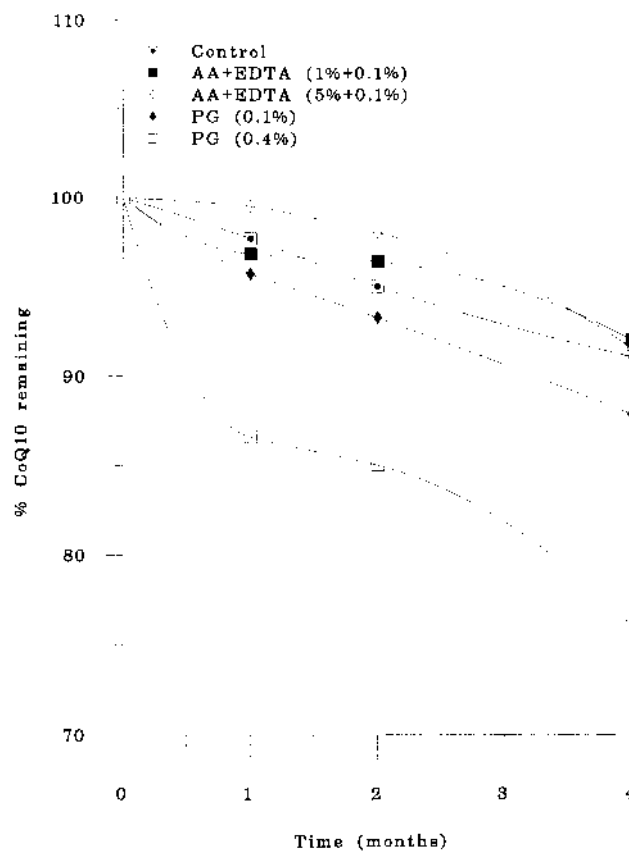Fig. 3. Stability of CoQ_{10} at Various Temperature Conditions

Fig. 4. Effect of Antioxidants on the Stability of CoQ_{10} at 55 °C

tions was observed. These variations may be attributed to differences in analytical techniques and/or species differences.

Following an oral dose of 60 mg per day for four days, the rise in plasma concentrations from the endogenous levels for formulation I and II were 0.51 ± 0.11 and $0.55\pm0.16\,\mu g/ml$, respectively. Such a small increase (2.5 fold from the base line) in plasma concentration reflects the poor absorption characteristics of CoQ_{10} with a reported half-life of 34 h.[2] Sanma *et al.* have reported similar results in a study with beagle dogs, where an increase in plasma concentrations of CoQ_{10} from 0.4 to 1.7 $\mu g/ml$ following oral administration of 10 mg/kg/d for four days was observed.[12] Further, poor absorption characteristics were also reported in humans. Langsjoen *et al.* reported mean plasma levels of 1.99 $\mu g/ml$, increased from 0.85 $\mu g/ml$, in patients with congestive heart failure following oral administration of 100 mg/d for 3 months.[13] In present studies, the T_{max} value was about 6 h and is in agreement with the reported value.[2] Poor and slow absorption characteristic of CoQ_{10} are attributed to its high molecular weight and high lipophilicity.[2]

Highly lipophilic compounds including CoQ_{10} are shown to be absorbed mainly by the route of intestinal lymphatics.[14,15] The absorption of these compounds depend on lipoprotein synthesis by the enterocyte. It is, therefore, advantageous to administer these drugs formulated in oils/lipids.[16] In the present investigations, the oil based formulation (II) showed slightly higher, but statistically not significant, bioavailability than the powder filled capsule. Since surfactants/emulsifiers are absent in the oil-based formulation (II), it did not form fine emulsion upon dilution with

water, which otherwise could have provided greater surface area for drug absorption. Incorporation of emulsifiers in the oil based formulations have been reported to improve the oral absorption of CoQ_{10}.[5,17] However, in contrast, Weis *et al.* reported higher bioavailability with soyabean oil supension of CoQ_{10} without any emulsifiers in comparison with similar formulations containing polysorbate 80 and/or lecithin.[4] These observations, may be explained on the basis of formation of micelles or mixed micelles in the intestine with the formulations containing emulsifiers which subsequently decreased the solubilization with bile salts.[4]

Stability Studies Effect of Temperature and Humidity: Glass vials containing CoQ_{10} were wrapped with aluminum foil and kept at various temperature and humidity conditions. The degradation profiles and the first-order rate constants are shown in Fig. 3. Degradation to a large extent was observed at 45 °C and 55 °C while it is relatively stable at 37 °C. Degradation rate constant at room temperature, calculated from the Arrhenius plot of $\log K$ *vs.* $1/T$ (not shown) was found to be 0.0014 month^{-1}. The predicted shelf life at room temperature (time to 90% potency) was 6.3 years. However, the effects of light, excipients, and formualtion processing conditions were not considered in these observations which otherwise might have affected the degradation. Our preliminary studies indicated that some of the commercially available products are not stable during their shelf life (unpublished results). Humidity conditions (11% RH, 51% RH, 91% RH) at 37 °C did not cause significant degradation (data not shown) over a period of 12 months.

Effect of Antioxidants at 55 °C: Samples of CoQ_{10} with
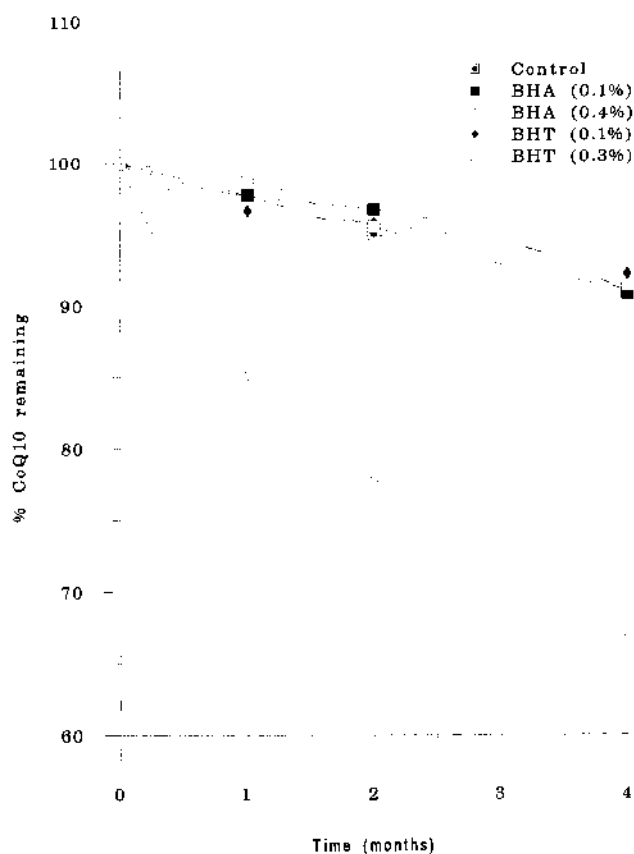
Fig. 5.   Effect of Phenolic Antioxidants on the Stability of CoQ_{10} at 55 °C
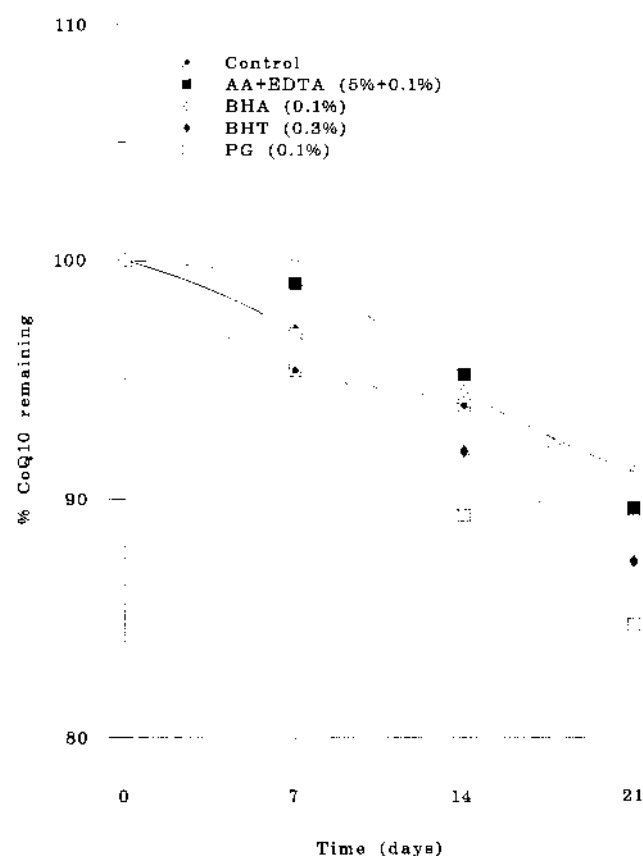

Fig. 6.   Effect of Antioxidants on the Photo Stability of CoQ_{10} (at 600 fc, 25 °C)

various antioxidants were taken in clear glass vials and kept at 55 °C. The degradation profiles are shown in Figs. 4 and 5. The antioxidant mixtures of AA and EDTA at 1—5% range and 0.1% were investigated. It was found that higher concentration of AA (5%) offered better protection than at lower concentration (1%) (Fig. 4). Chelating agents are usually employed in the concentration range of 0.005—0.1% to enhance the stability and also to inhibit possible pro-oxidation effects, especially in presence of trace metal ions, by AA.[18,19] The inhibition of autoxidation by AA can be explained by several mechanisms. In general, in the presence of metal ions, ascorbate monoanion reacts rapidly with oxygen and forms ascorbate radical which further oxidizes to dehydroascorbic acid. This is usually considered the primary mechanism for AA, especially in closed systems.[19] In addition, AA can also act as a chain breaking antioxidant since it can form a stable free radicals.[19]

PG at 0.1% level increased the degradation of CoQ_{10} compared to control and an increase in PG concentration from 0.1 to 0.3% further accelerated the degradation process (Fig. 4). This pro-oxidation effect of PG may be explained as follows. During the oxidation process, antioxidants convert the activated drug molecules (free radicals) to thermodynamically less active or inactive forms. The radical antioxidants formed in this process react with one another and terminate the reaction, but under normal circumstances they do not react with the drug molecules. However, if the antioxidant radicals are reactive towards substrate, then autoxidation of substrate accelerates. This reaction is unlikely for stable phenolic radicals especially those with bulky groups around the

radical center.[19] In PG, the radical center is not surrounded by bulky groups, as in cases of BHT and BHA and, therefore, may be more reactive towards CoQ_{10} molecules leading to pro-oxidation effects. In additon, BHT in the concentration range of 0.1—0.3% and BHA at 0.1% offered better protection (Fig. 5). However, increasing concentrations of BHA from 0.1% to 0.4% accelerated the degradation of CoQ_{10}. When an antioxidant is present in excess, it may promote acceleration rather than inhibition by promoting the decomposition of peroxides which in turn promote the oxidation process.[7]

Photostability studies: The fluorescent light (typically 400—800 lx) provides reasonable simulation of natural light and widely used in photostability testing.[20] Samples of CoQ_{10} with various antioxidants were kept in clear glass vials and exposed to fluorescent light (600 lx at 25 °C) in a light stability chamber. The degradation profiles are shown in Fig. 6. The antioxidant mixture of AA (5%) and EDTA (0.1%) offered better protection than other antioxidants such as BHA (0.1%), BHT (0.3%), or PG (0.1%). AA in the concentrations of 1—5% and in combination with EDTA (0.1%) offered protection to the same extent (data not shown). Further, increasing concentrations of BHA and PG from 0.1 to 0.3% did not improve the photostability (data not shown). In Fig. 6, increase in degradation rate can be observed in all the profiles after a 7-day period. This period is the lag phase or induction period during which sufficient concentration of the free radicals build-up to propagate the reaction.[7] The length of the induction period varies depending on the an-

tioxidant(s) used and the stability of the compound.[7]

In conclusion, bioavailability of powder filled capsule and oil-based formulations of CoQ_{10} are comparable ($p<0.95$). Poor and slow absorption of CoQ_{10} were observed for both the formulations. Following oral administration of 60 mg/d for four days, the mean plasma concentrations were raised by 0.51 and 0.55 μg/ml for formulation I and II, respectively. In stability studies of CoQ_{10}, large extent of degradation was observed at 45 °C and 55 °C, and humidity conditions did not affect the stability of CoQ_{10}. Among the various antioxidants evaluated, mixture of AA (5%) and EDTA (0.1%) offered better protection of CoQ_{10} when exposed to light (600 lx, 25 °C) or to a higher temperature (55 °C). Increasing concentrations of phenolic antioxidants (from 0.1% to 0.3%) such as BHA and PG accelerated the degradation of CoQ_{10}. Stable formulations of CoQ_{10} may be developed incorporating ascorbic acid (5%) and EDTA (0.1%) as an antioxidant system.

References

1) Folkers K., Wolaniuk A., Vadhanavikit S., Sakmato N., Takemura K., Baker L., Richardson P. C., "Biomedical and clinical aspects of Coenzyme Q," Vol. 5, ed. by Folkers K., Yamamura Y., Elsevier, Amsterdam, 1986, pp. 375—391.
2) Greenberg S., Fishman W. H., *J. Clin. Pharmacol.*, **30**, 590—608 (1990).
3) Kishi H., Kanamori N., Nishii S., Hiraoka E., Okamato T., Kishi T., "Biomedical and clinical aspects of Coenzyme Q," ed. by Folkers K., Yamamura Y., Chapter 3, Elsevier Science, Amsterdam, 1984, pp. 131—142.
4) Weis M., Mortensen S. A., Rassing M. R., Moller-Sonnergaard J., Poulsen G., Rasmussen S. N., *Mol. Aspects Med.*, **15**, S273—S278 (1994).
5) Chopra R. K., Goldman R., Sinatra S. T., Bhagavan H., *Int. J. Vit. Nutr. Res.* **68**, 109—113 (1998).
6) Matsuda Y., Masahara R., *J. Pharm. Sci.*, **72**, 1198—1203 (1983).
7) Chalmers L., Antioxidants: Research and development report. S.P.C. (1971) 29—38.
8) Kommuru T. R., Khan M. A., Ashraf M., Kattenacker R., Reddy I. K., *J. Pharm. Biomed. Anal.*, **16**, 1037—1040, (1998).
9) Kaikkonen J., Nyssonen K., Porkkala-Sarataho E., Poulsen H. E., Metsa-Ketela T., Hayn M., Salonen R., Salonen J. T., *Free Rad. Biol. Med.*, **22**, 1195—1202 (1997).
10) Bogentoft C., Edlund P. O., Olsson B., Wilund L., Westensen K., "Biomedical and clinical aspects of Coenzyme Q," Chapter 6, ed. by Folkers K., Yamamura Y., Elsevier, Amsterdam, 1991, pp. 215—224.
11) Tomono Y., Hasegawa J., Seki T., Motegi K., Morishita N., *Int. J. Clin. Pharmacology, Therapy and Toxicology*, **24**, 536—541 (1986).
12) Sanma H., Yamada H., Yamano Y., Tanaka M., Tajima T., Kanazawa T., Hori S., Nakazawa H., Yoshino H., Miyamori R., Kojima M., Nakamura Y., "Biomedical and Clinical Aspects of Coenzyme Q," Vol. 5, ed. by Folkers K., Yamamura Y., Elsevier, Amsterdam,1986, pp. 99—100.
13) Langsjoen P. H., Langsjoen P., Folkers K., *Am. J. Cardiol.*, **65**, 521—523 (1989).
14) Stella V. J., Charman W. N. A., *Int. J. Pharm.*, **34**, 175—178 (1986).
15) Aso Y., Mishima K., Arisaka T., Kitagawa H., Abstracts of Papers, Ist Symposium on Drug Metabolism and Action, Chiba, November 1969, p. 4-(1).
16) Palin K. J., Wilson C. J., Davis S. S., Philips A. J., *J. Pharmcol.*, **34**, 707—710 (1982).
17) Katayama K., Fujita T., *Chem. Pharm. Bull.*, **20**, 2585—2592 (1972).
18) Handbook of Pharmaceutical excipients, American Pharmaceutical Association, Washington D.C., 1986, p. 176.
19) Johson D. M., Gu L. C., "Encyclopedia of Pharmaceutical Technology," Vol. 1, ed. by Swarbrick J., Boylan J. C., Marcel Dekker, NY, 1988, pp. 415—450.
20) Anderson N. H., Johnston D., Mclelland M. A., Munden P., *J. Pharm. & Biomed. Anal.*, **9**, 443—449 (1991).